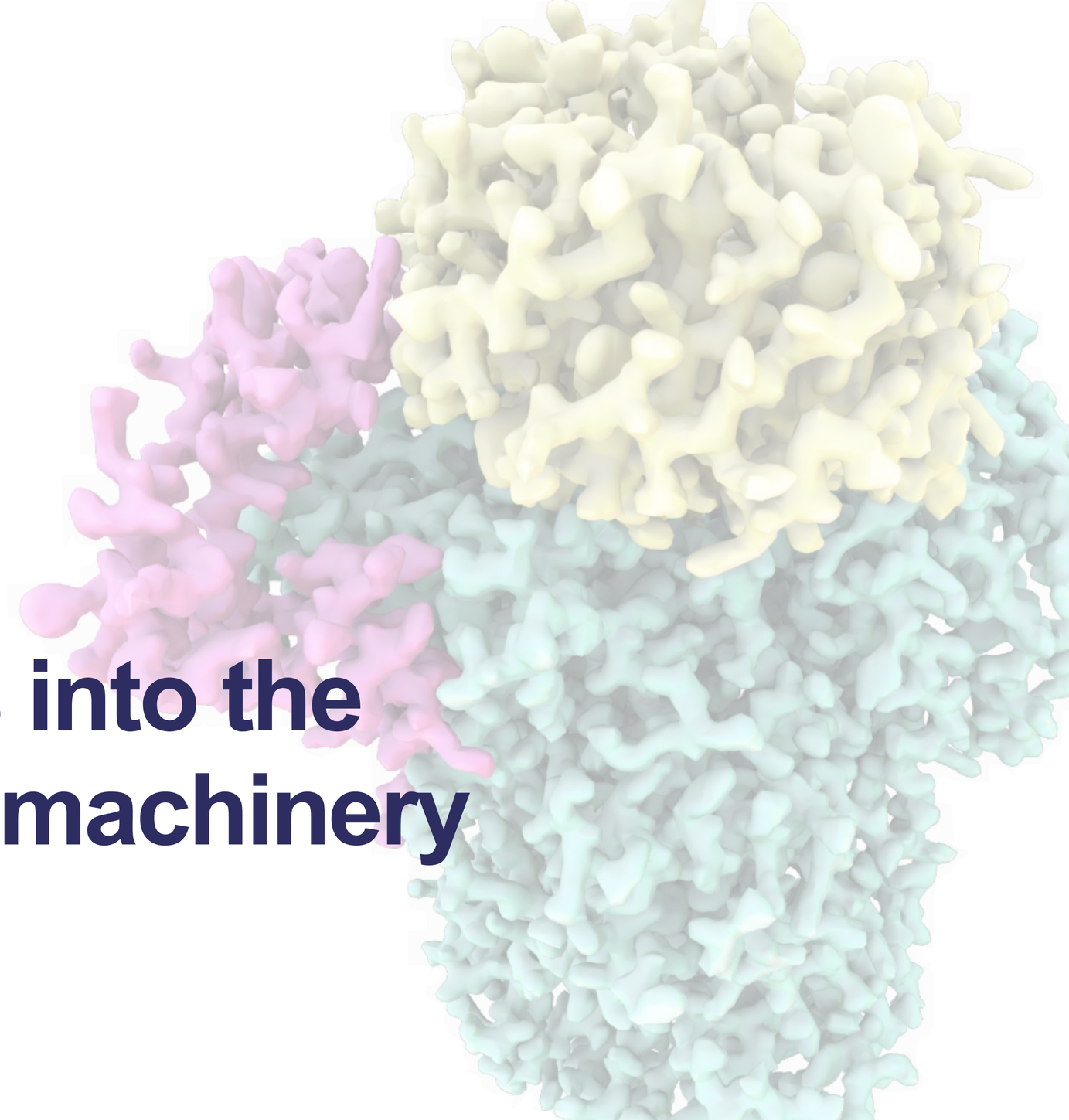
MRC Laboratory of Molecular Biology

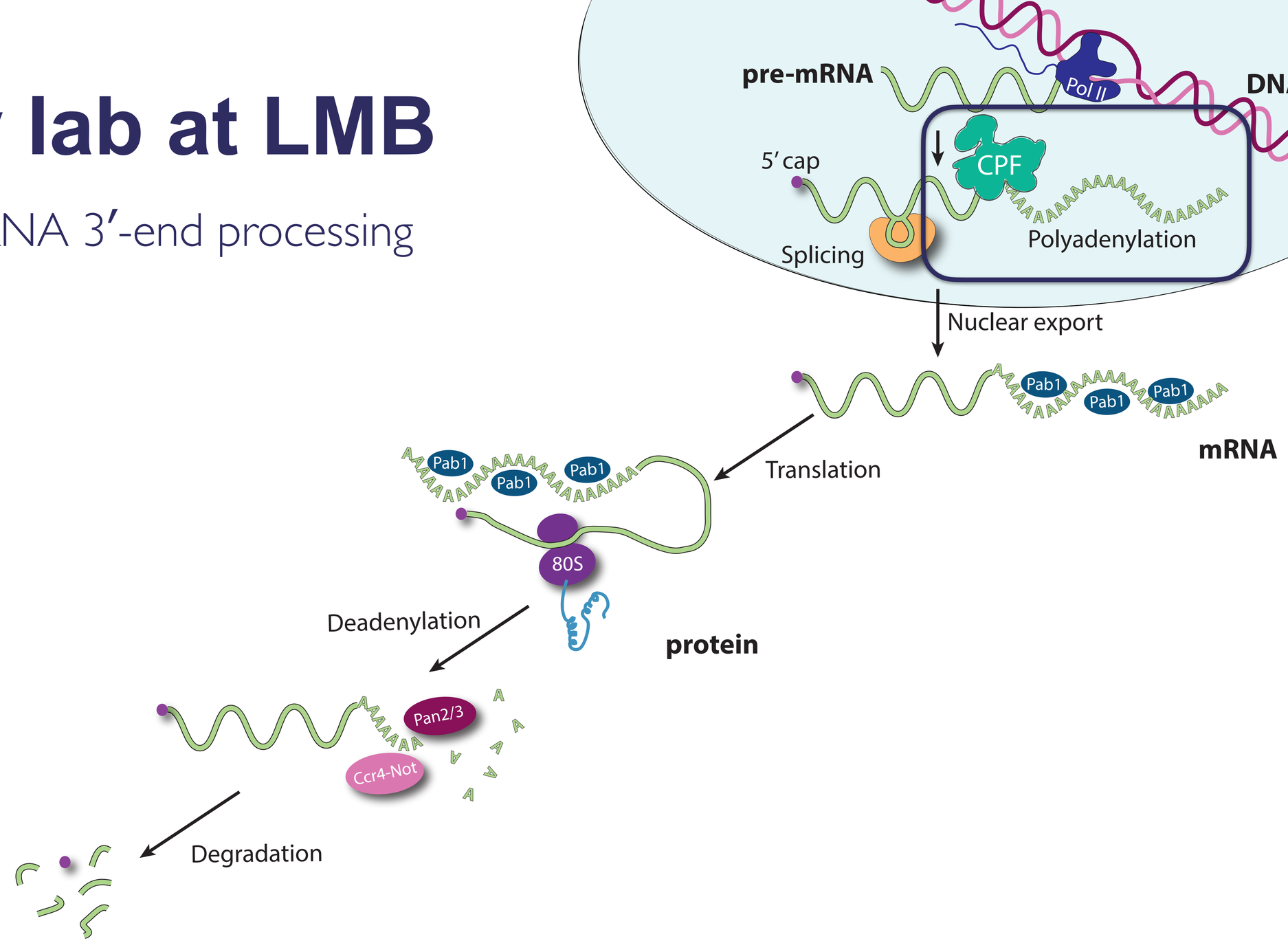


Molecular insights into the mRNA poly(A) tail machinery

Lori Passmore

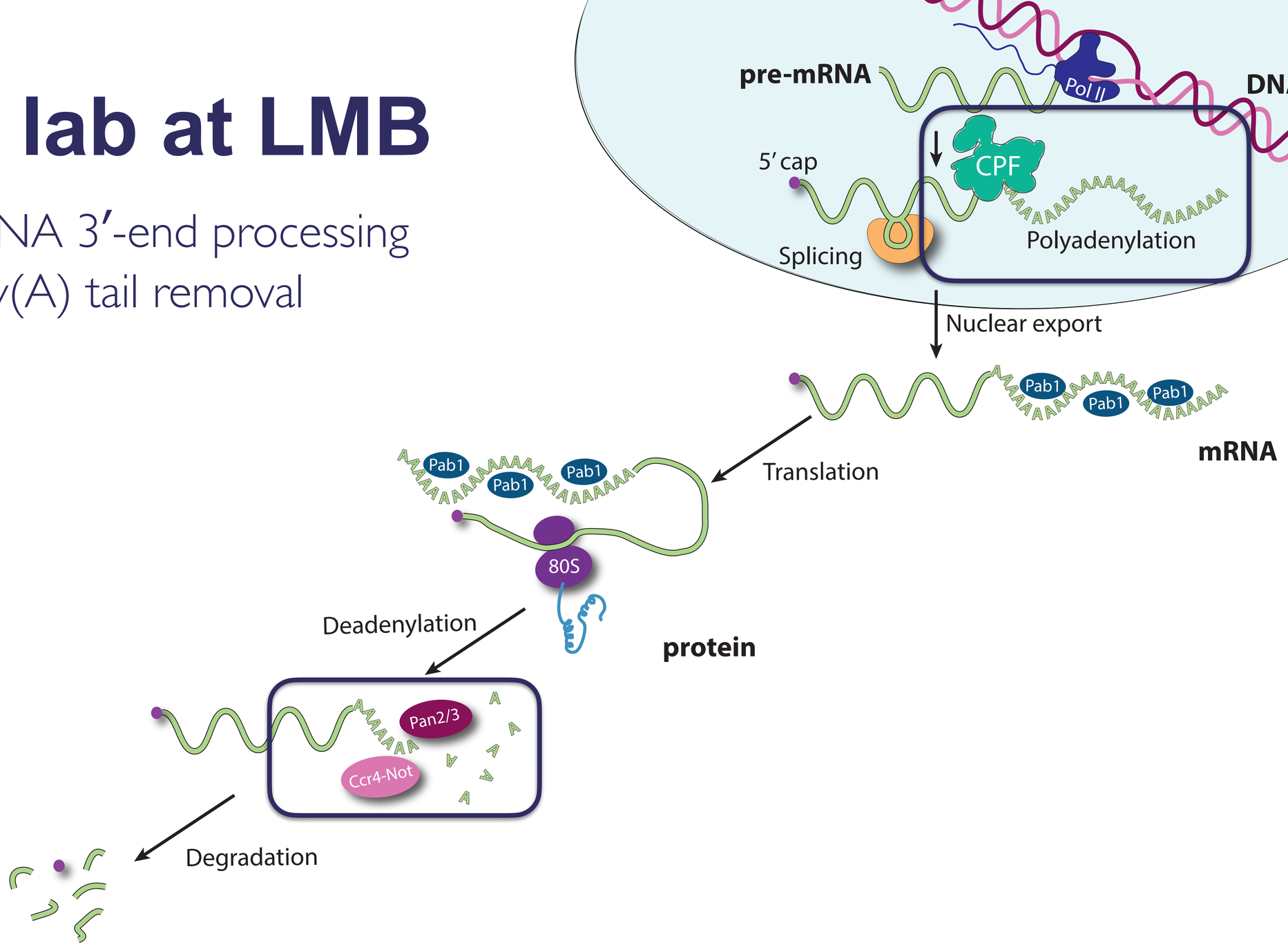
My lab at LMB

I. mRNA 3′-end processing



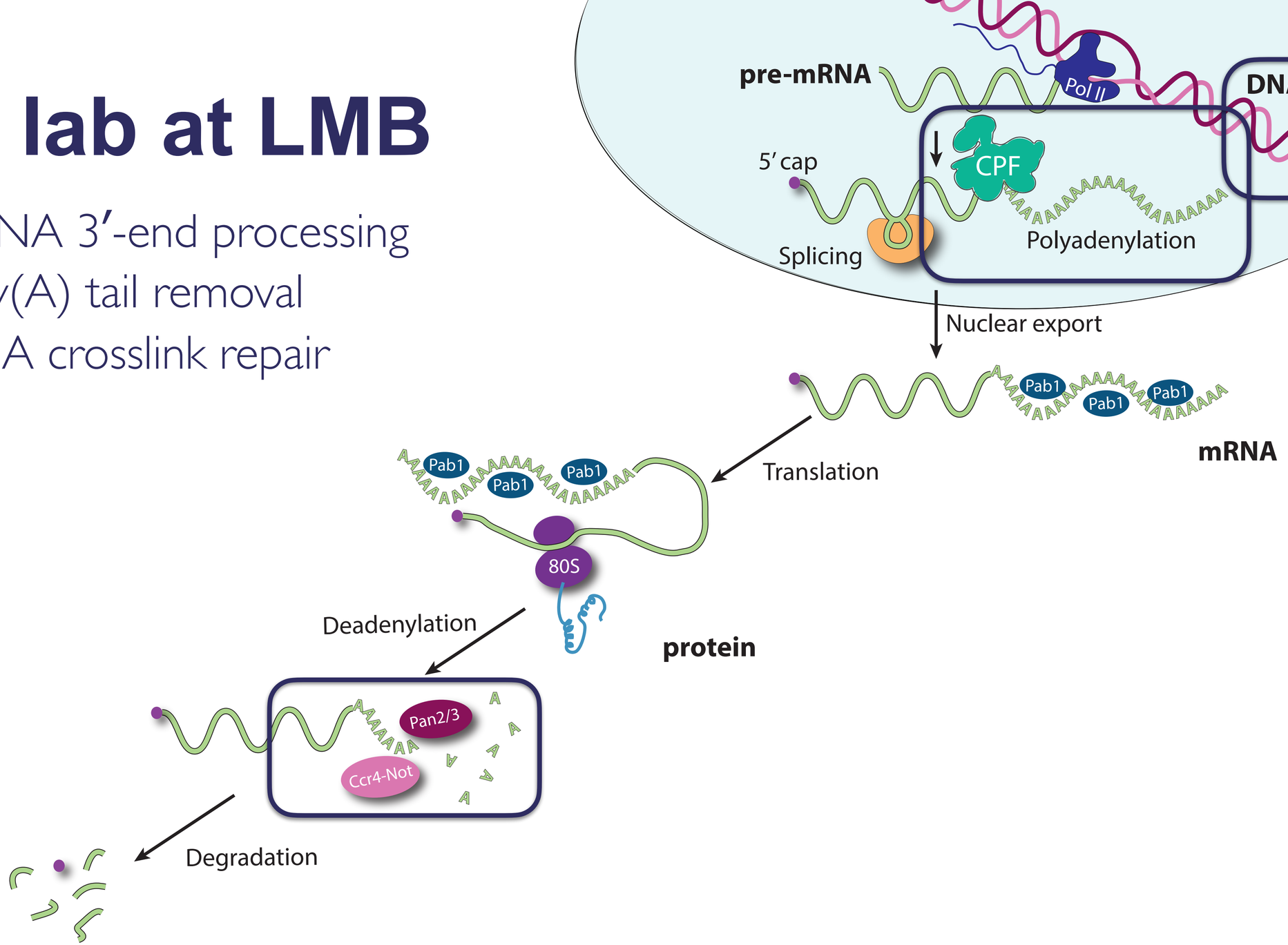
My lab at LMB

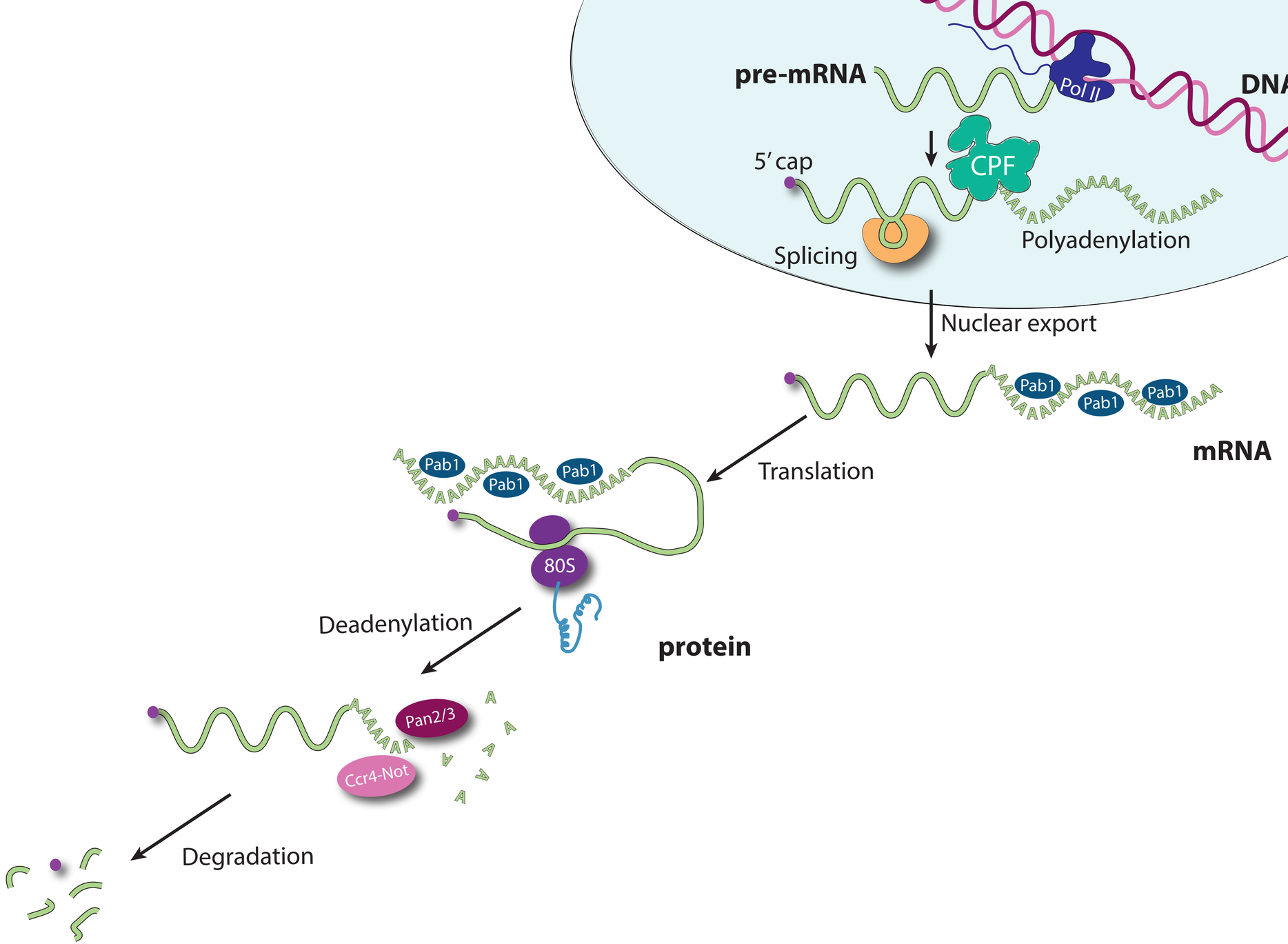
1. mRNA 3′-end processing
2. poly(A) tail removal

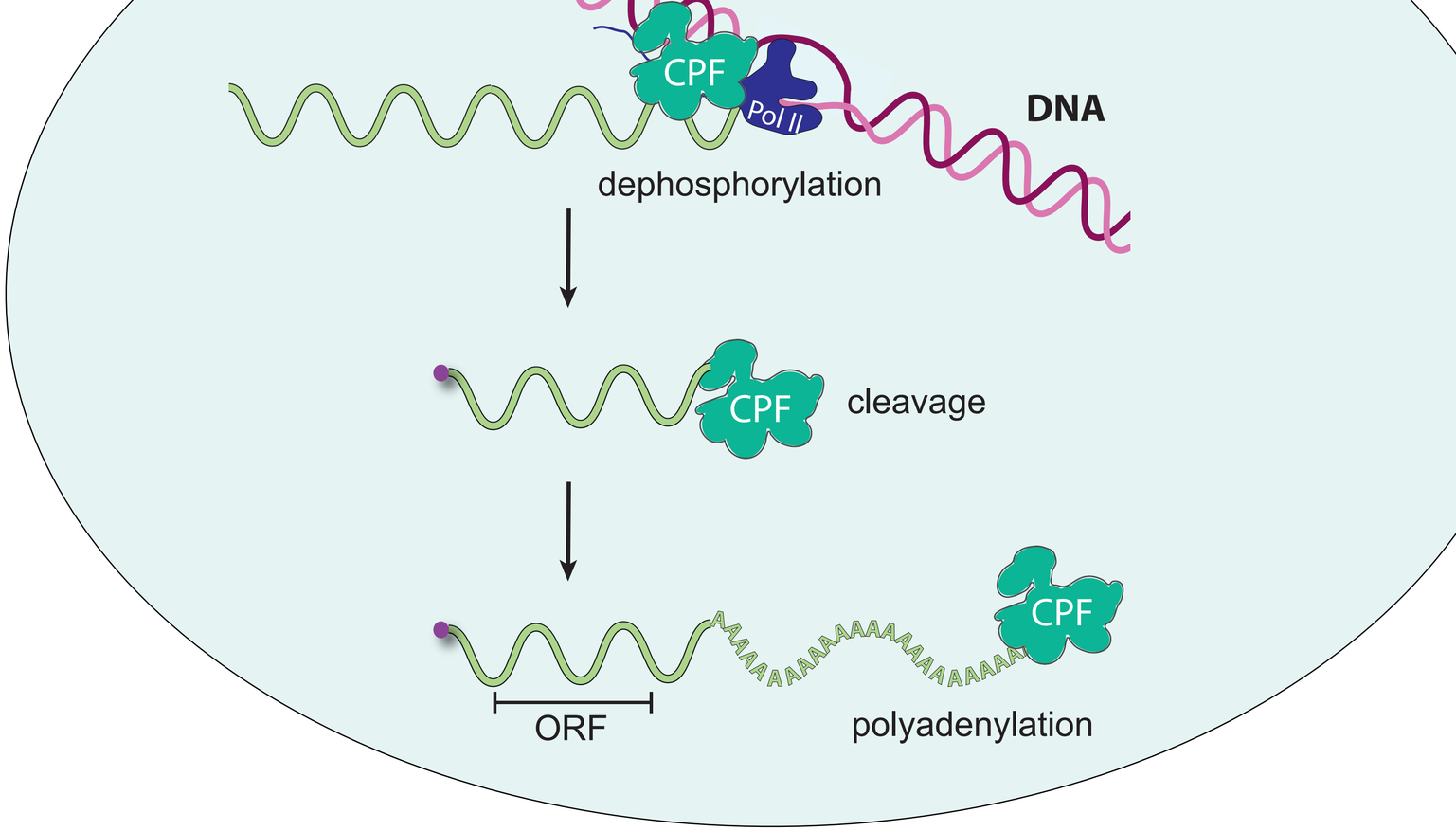


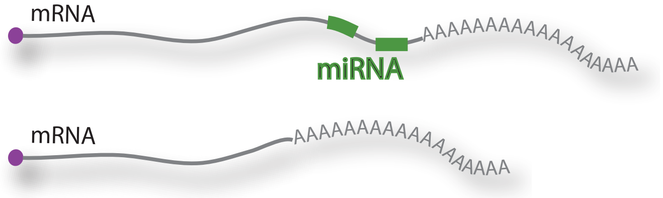
My lab at LMB

1. mRNA 3′-end processing
2. poly(A) tail removal
3. DNA crosslink repair



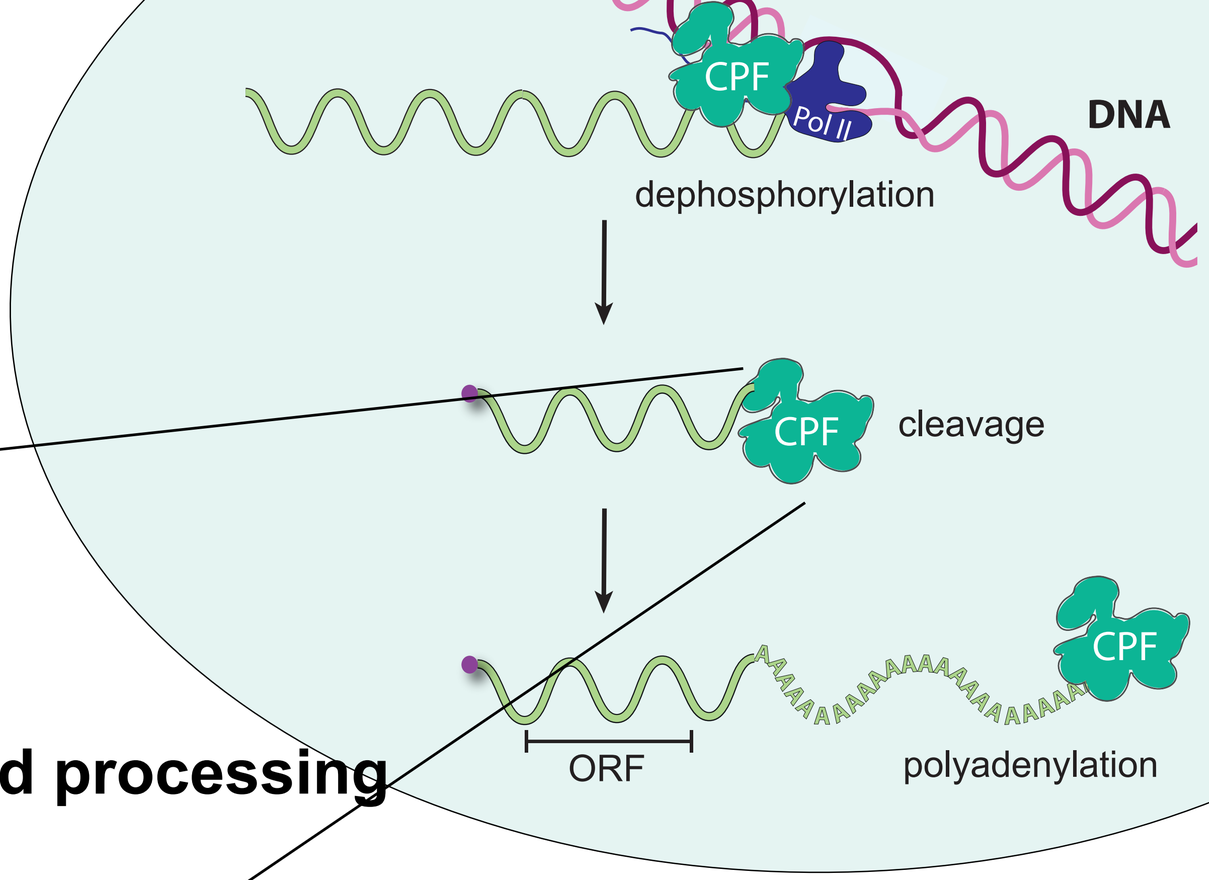

pre-mRNA
Pol II
DNA
5′ cap
CPF
Splicing
Polyadenylation
Nuclear export
Pab1
Pab1
Pab1
mRNA
Translation
80S
protein
Deadenylation
Pan2/3
Ccr4-Not
Degradation

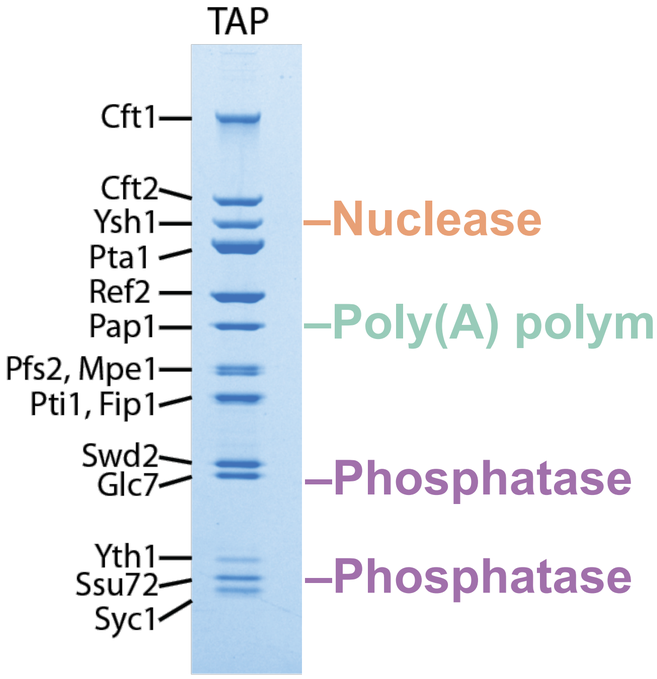




The length and position of poly(A) tails are important for gene regulation.

Globin mRNA polyA site mutations (LOF) - **thalassemia**
Prothrombin mRNA polyA site mutations (GOF) - **thrombophilia**
Alternative polyadenylation - **cancer**
Disruption of host polyadenylation - **influenza A**





–Nuclease

–Poly(A) polymerase

–Phosphatase

–Phosphatase

mRNA 3' end processing

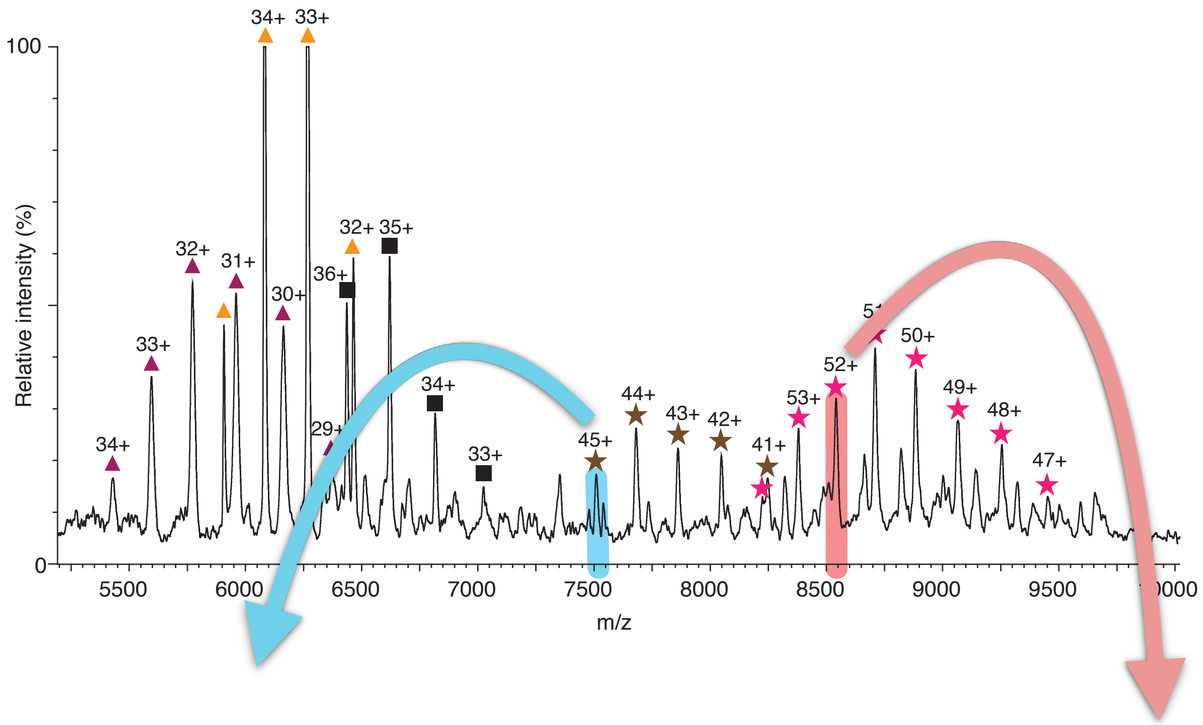
+

Transcription termination

Identification of enzymatic modules in CPF

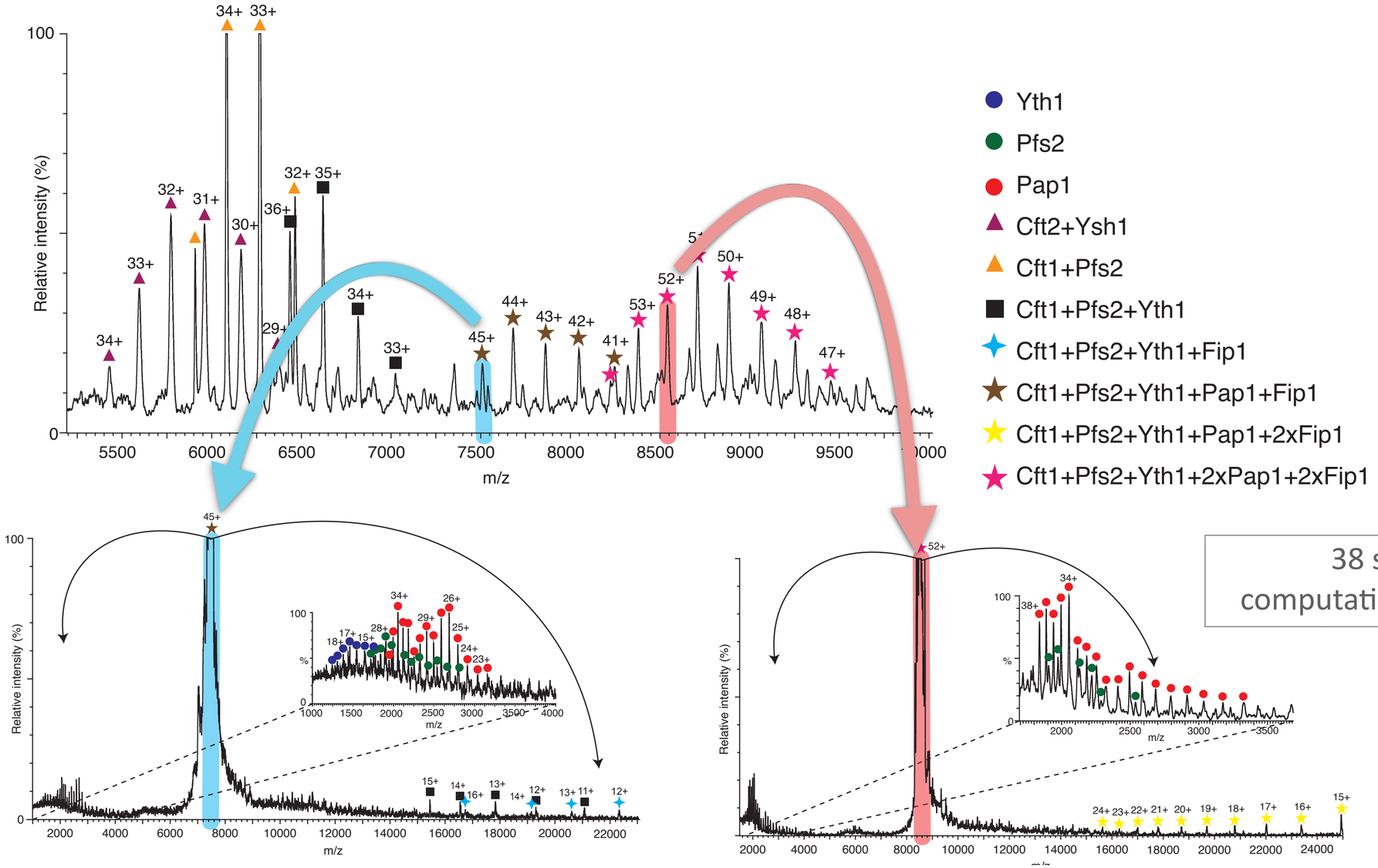
Ashley Easter, Yuliya Gordiyenko, Carol Robinson

Identification of enzymatic modules in CPF



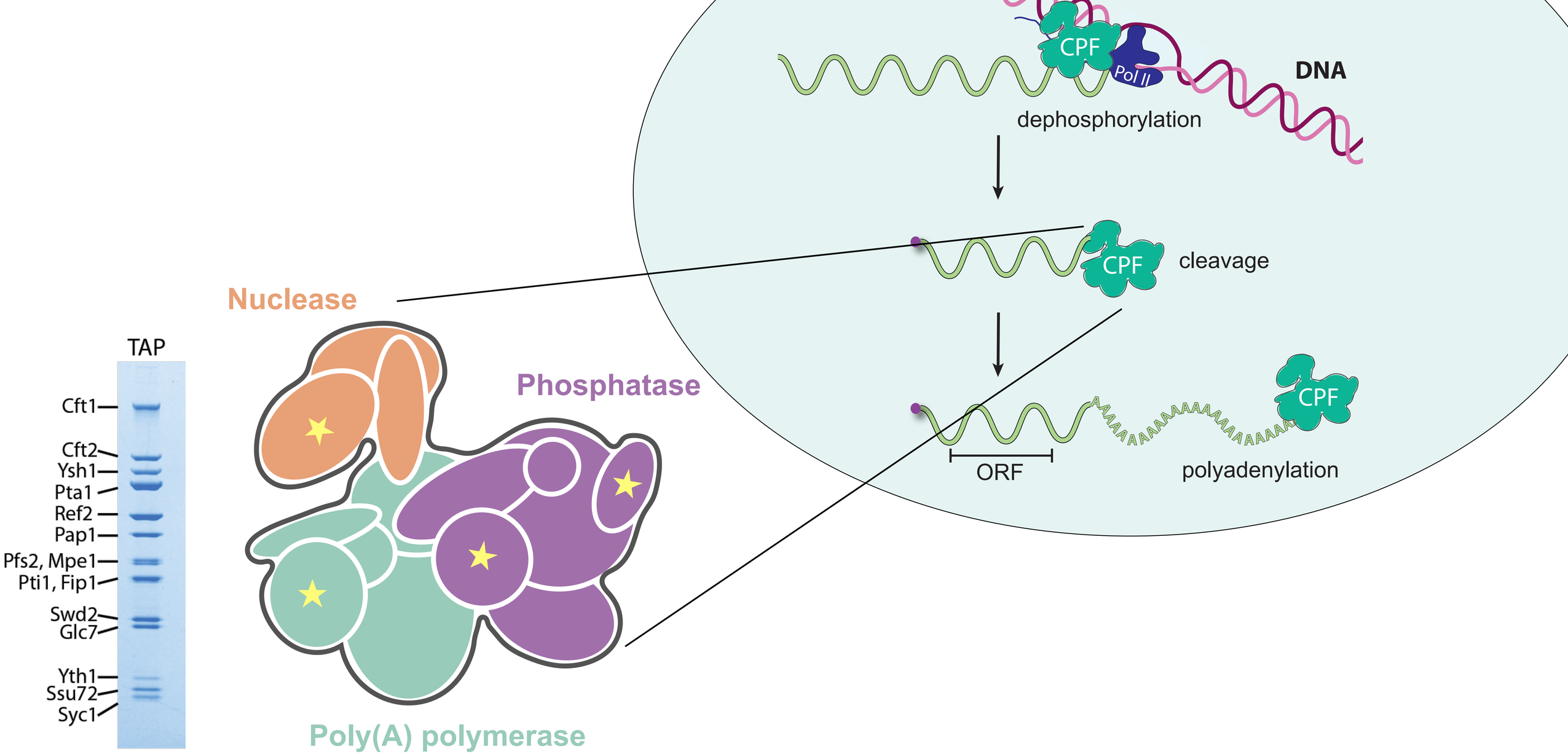
Ashley Easter, Yuliya Gordiyenko, Carol Robinson

Identification of enzymatic modules in CPF



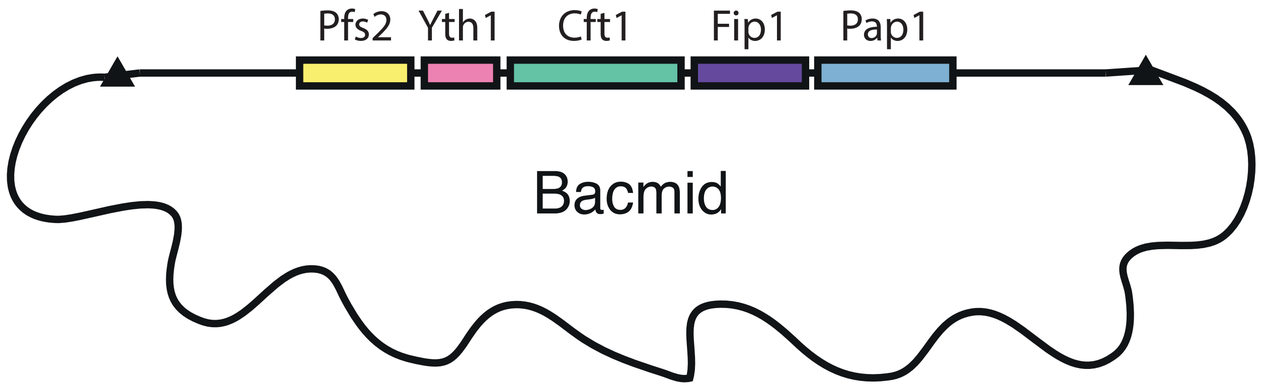
38 sub complexes,
computational network analysis

Ashley Easter, Yuliya Gordiyenko, Carol Robinson

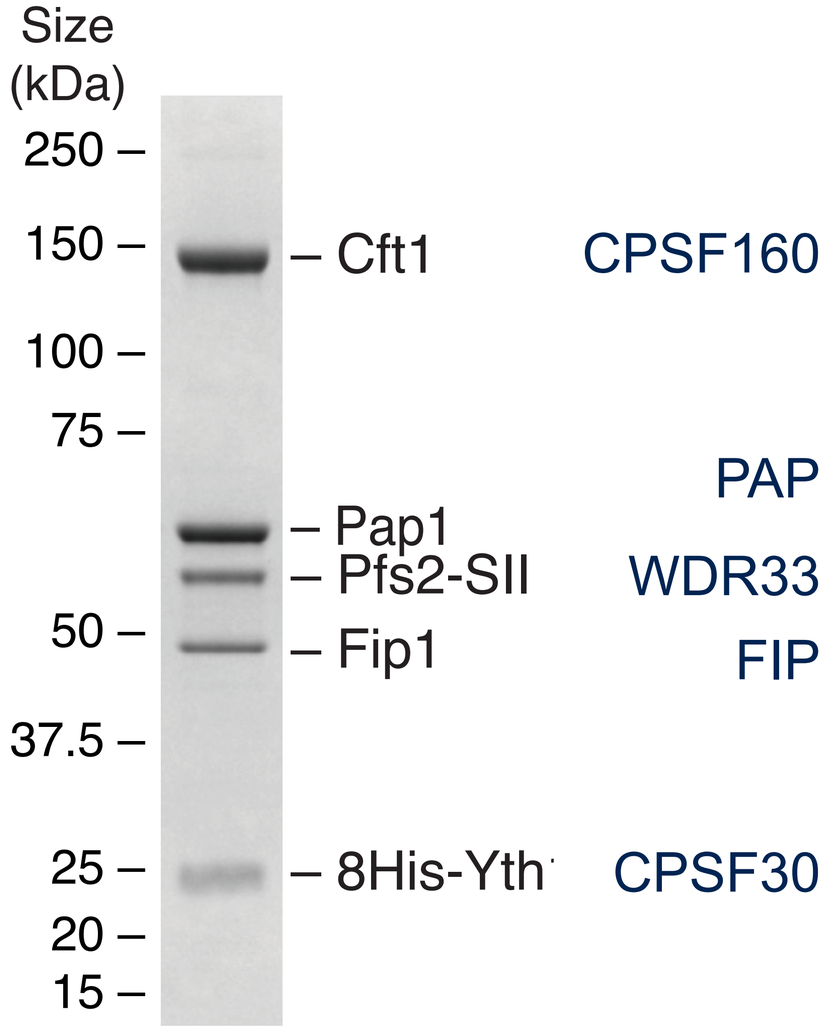


Casañal, Kumar et al, *Science* 2017 (with Carol Robinson)

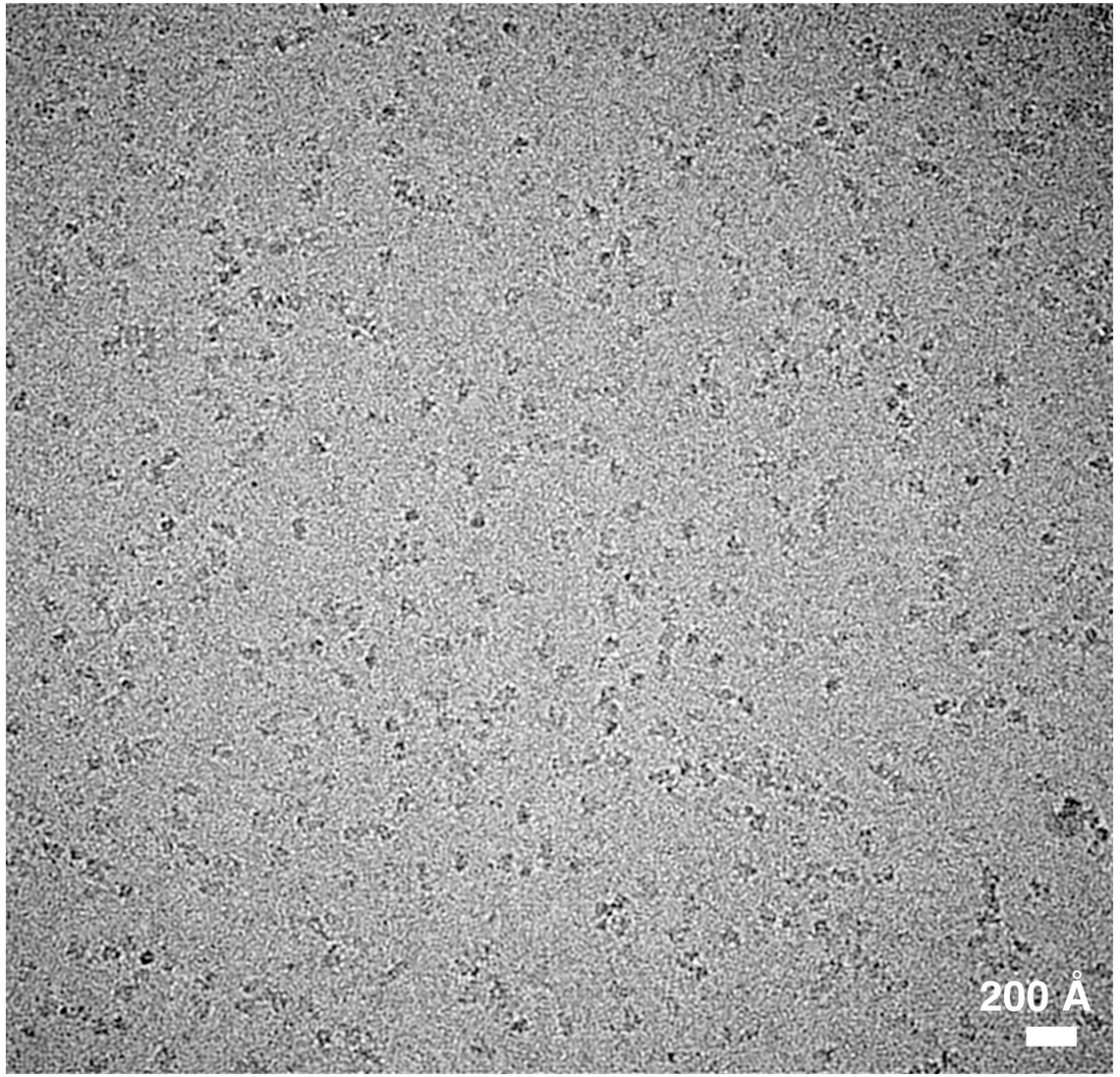
The polymerase module

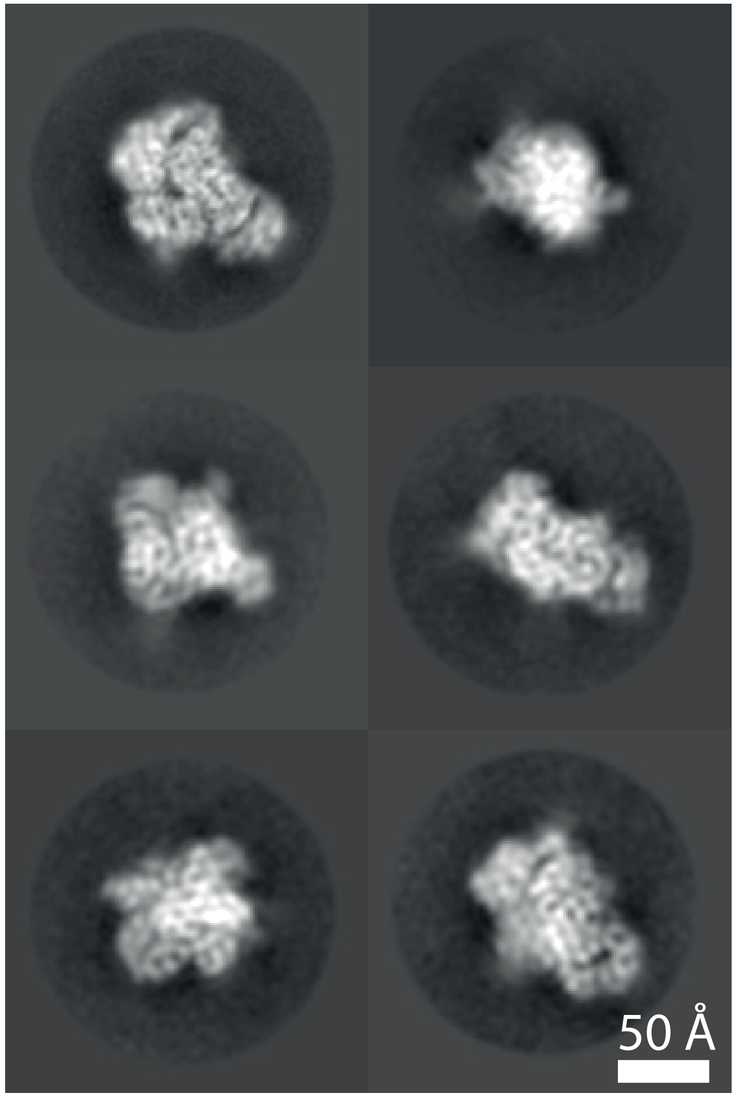


Ananth Kumar
Ana Casañal



The polymerase module



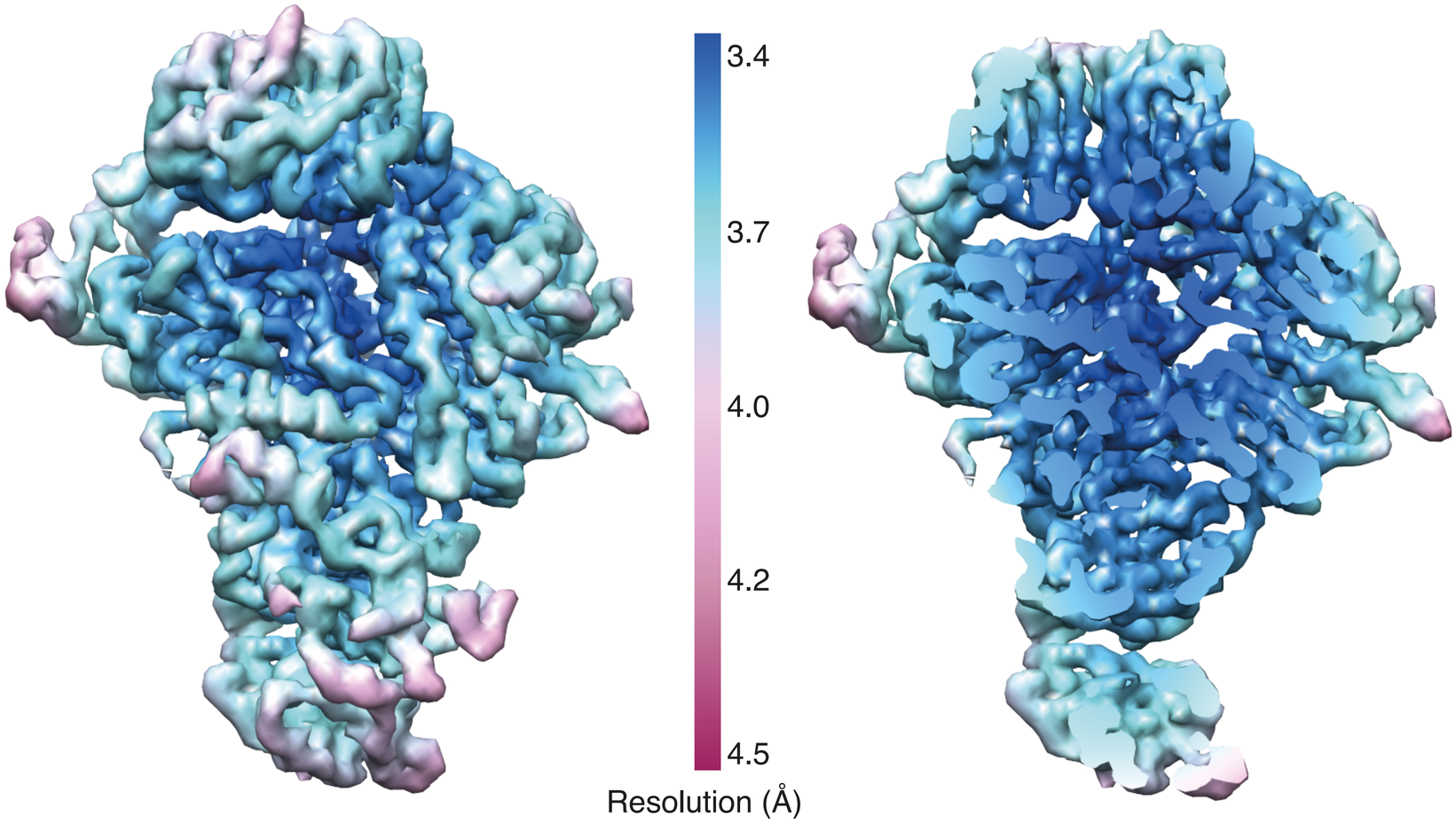


~ 200 kDa
No polymerase

UltrAuFoils
Titan Krios, K2 (LMB and eBIC)

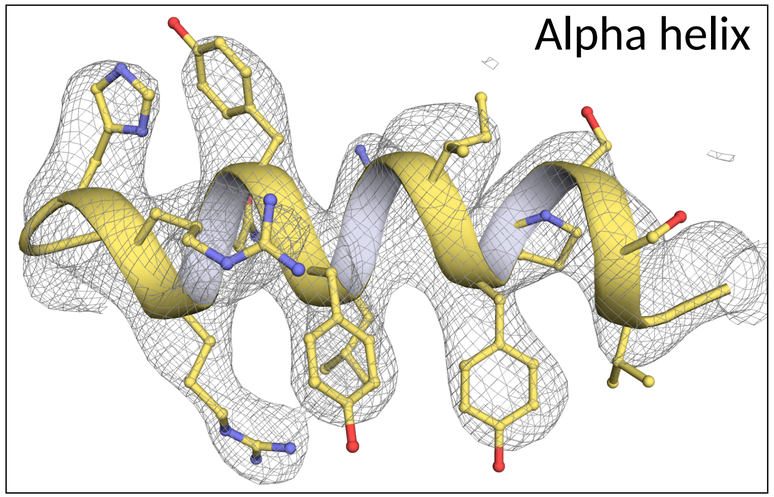
Ana Casañal, Ananth Kumar

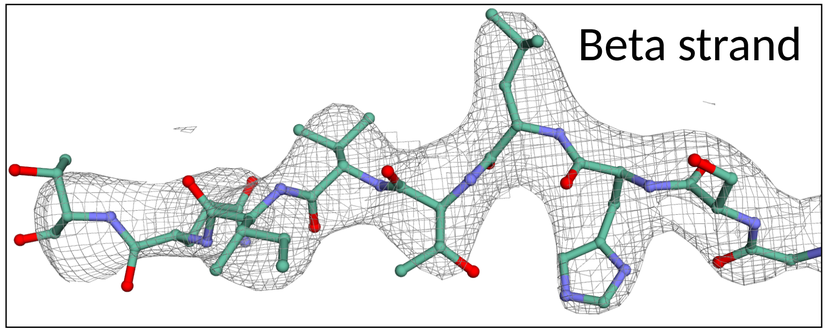
Cryo-EM of the polymerase module

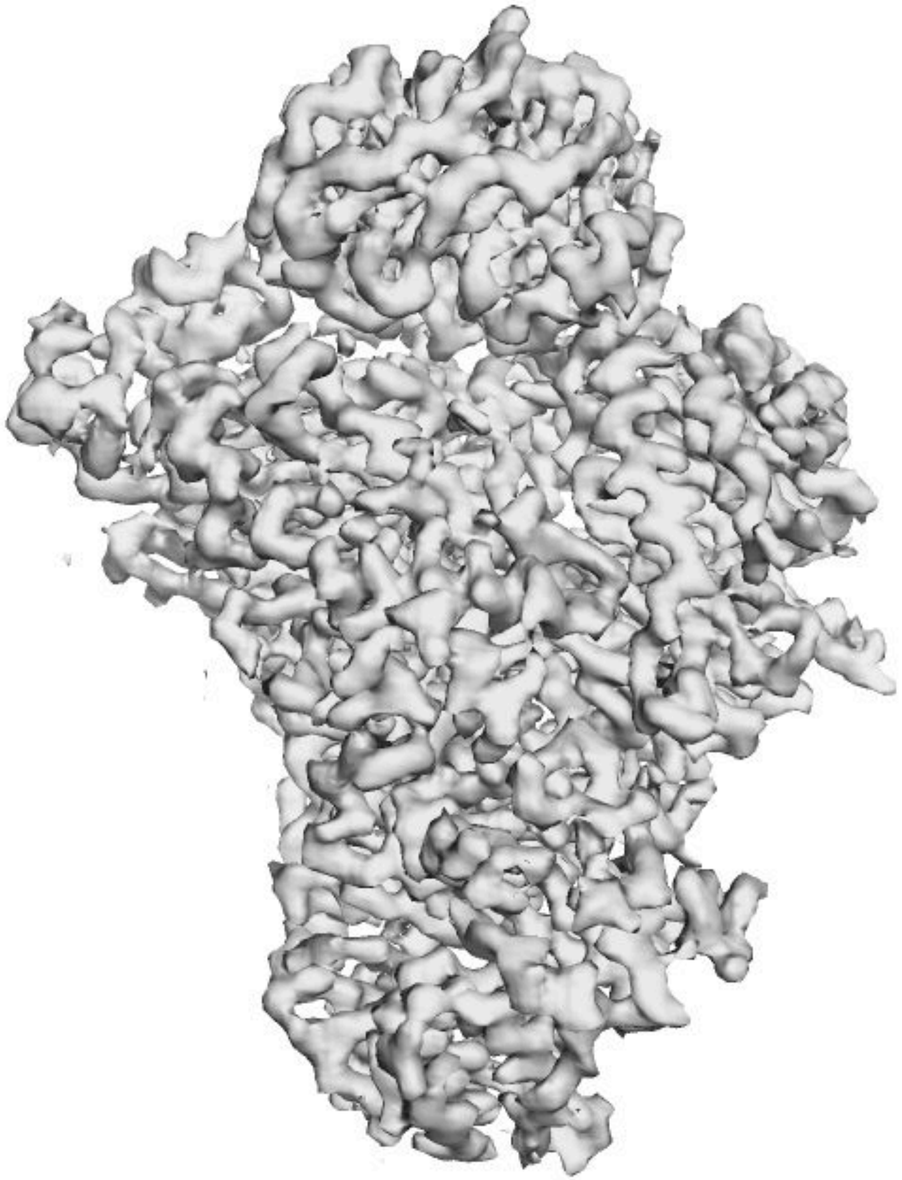


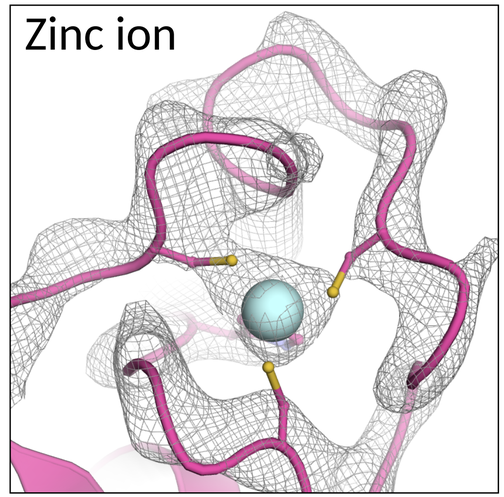
Relion 2
79,950 particles

The polymerase module





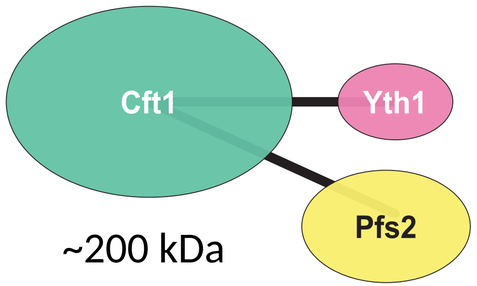




Ananth Kumar
Ana Casañal

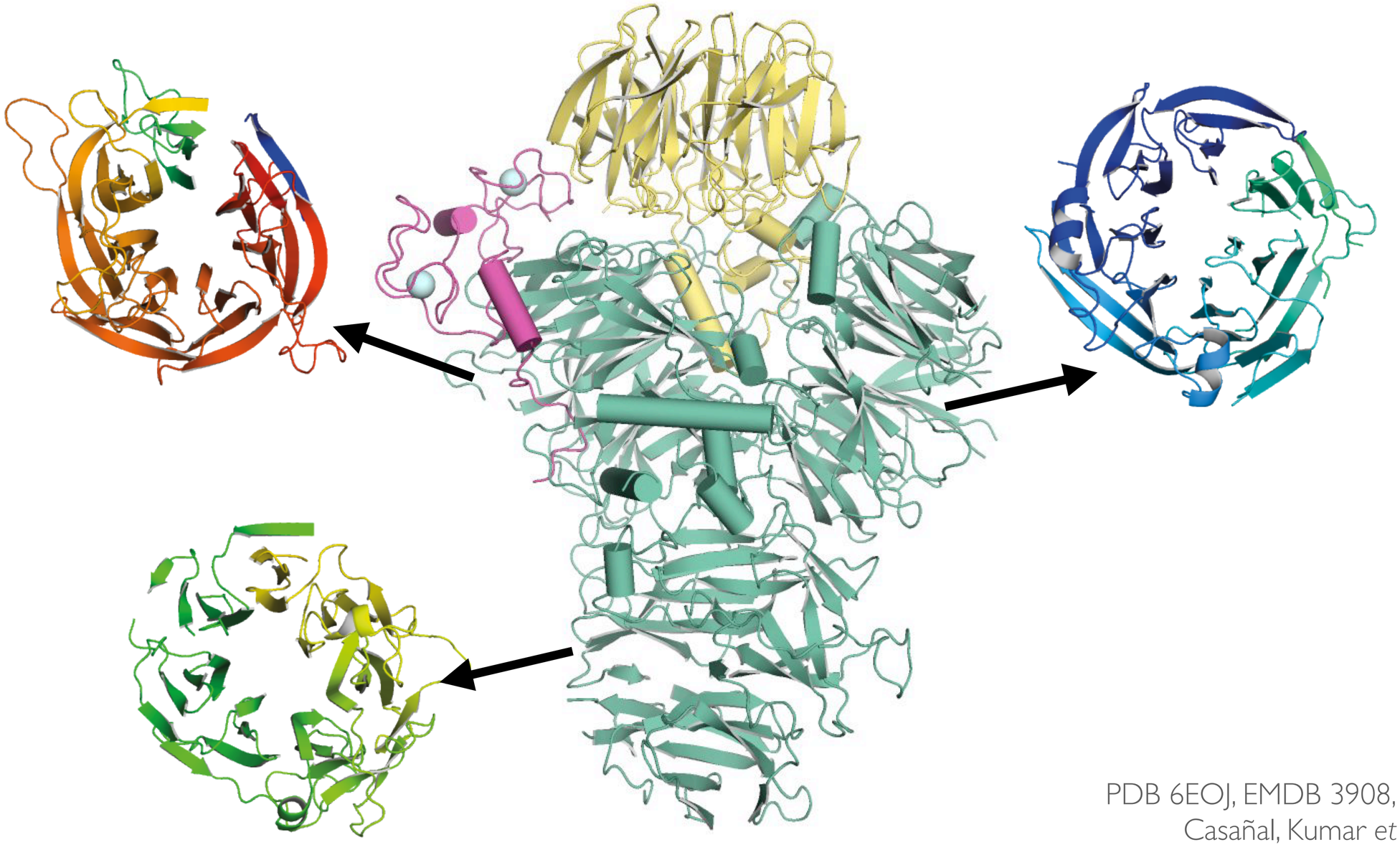
- 3.5 Å resolution overall
- 3D classification
- focussed classification for individual domains with heterogeneity/flexibility

The polymerase module



PDB 6EOJ, EMDB 3908, EMPIAR 10299
Casañal, Kumar *et al* *Science* 2017

The polymerase module

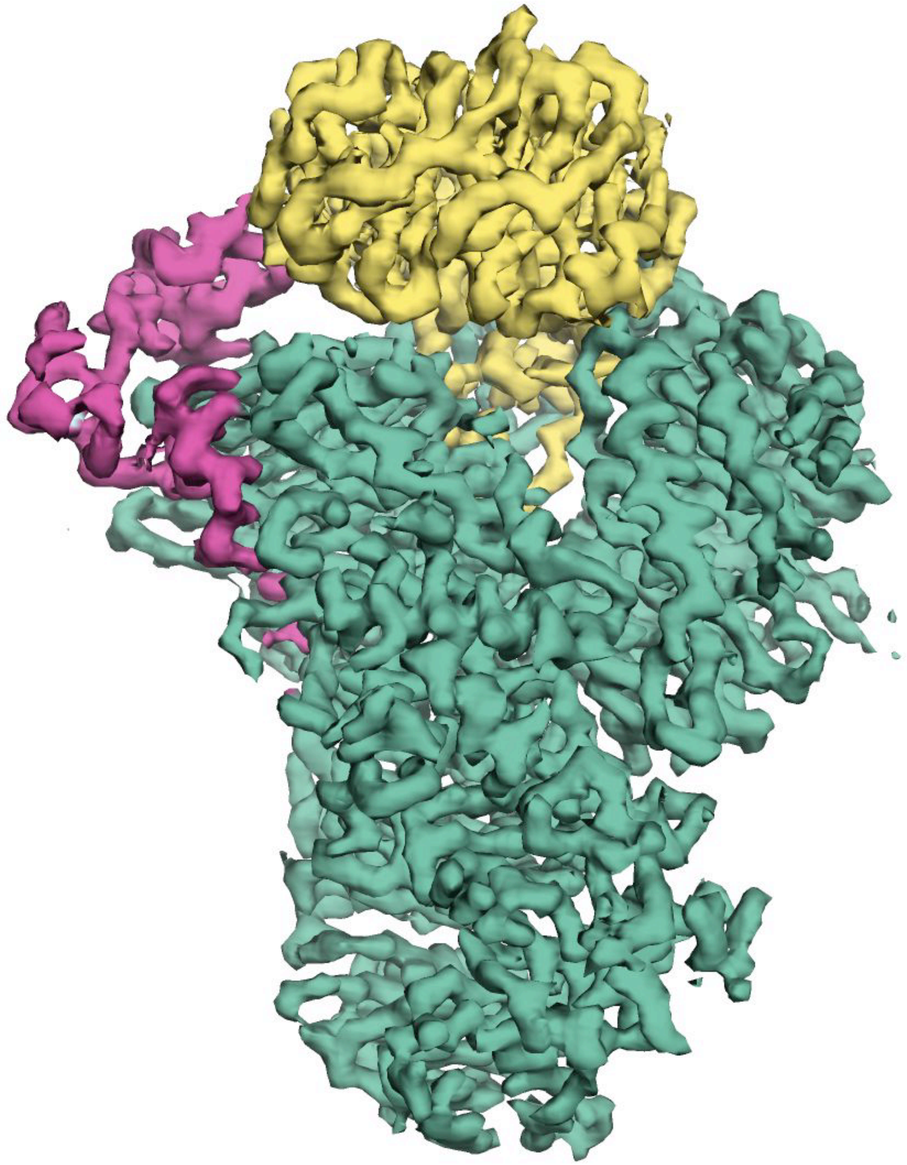


PDB 6EOJ, EMDB 3908, EMPIAR 10299
Casañal, Kumar *et al* *Science* 2017

Interaction of Yth1 with the polymerase module

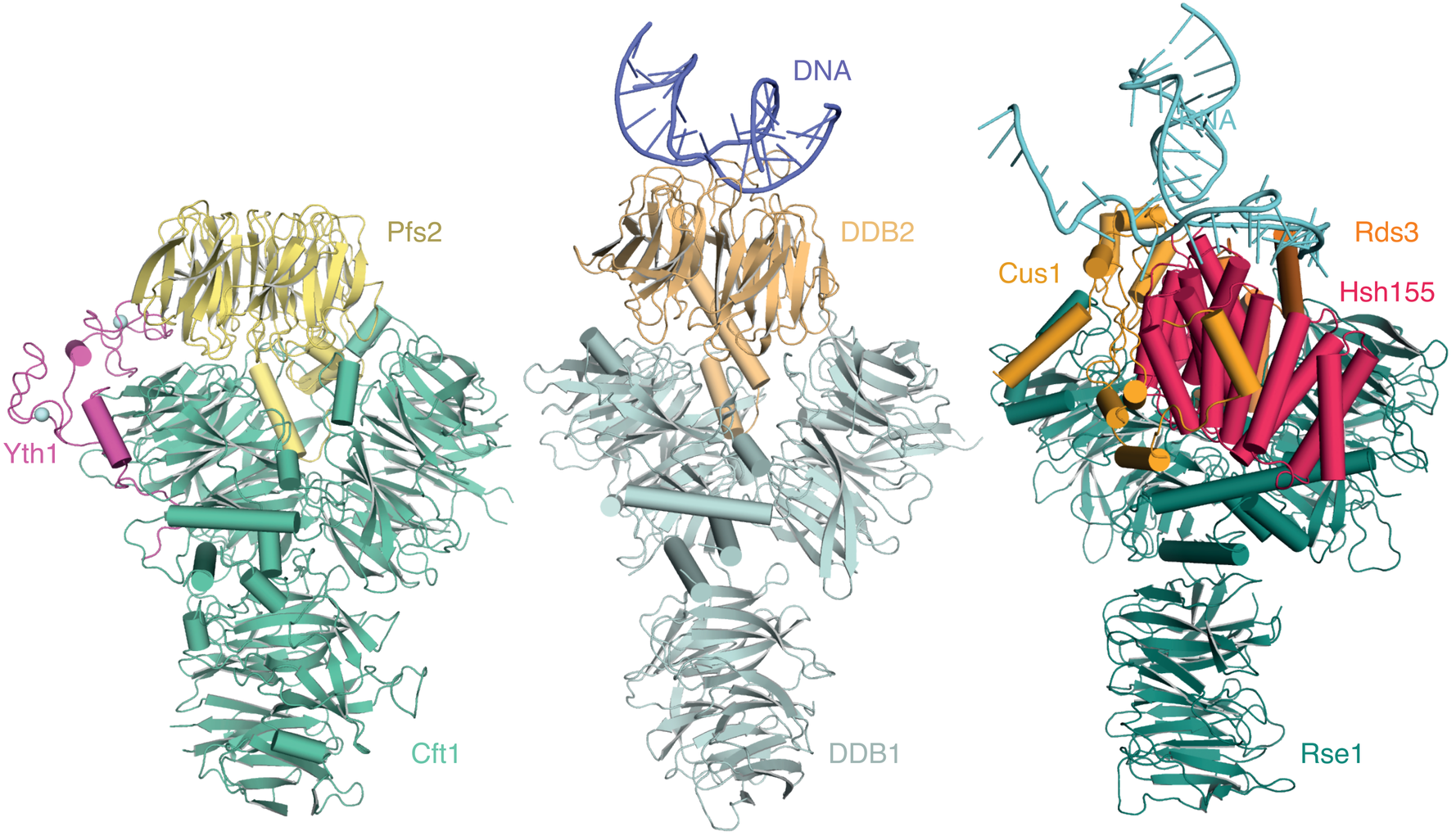
PDB 6EOJ, EMDB 3908, EMPIAR 10299
Casañal, Kumar *et al* *Science* 2017

Interaction of Pfs2 with polymerase module



PDB 6EOJ, EMDB 3908, EMPIAR 10299
Casañal, Kumar *et al Science* 2017

Similarity to other nucleic acid binding complexes



mRNA 3′ end processing
CPF polymerase module

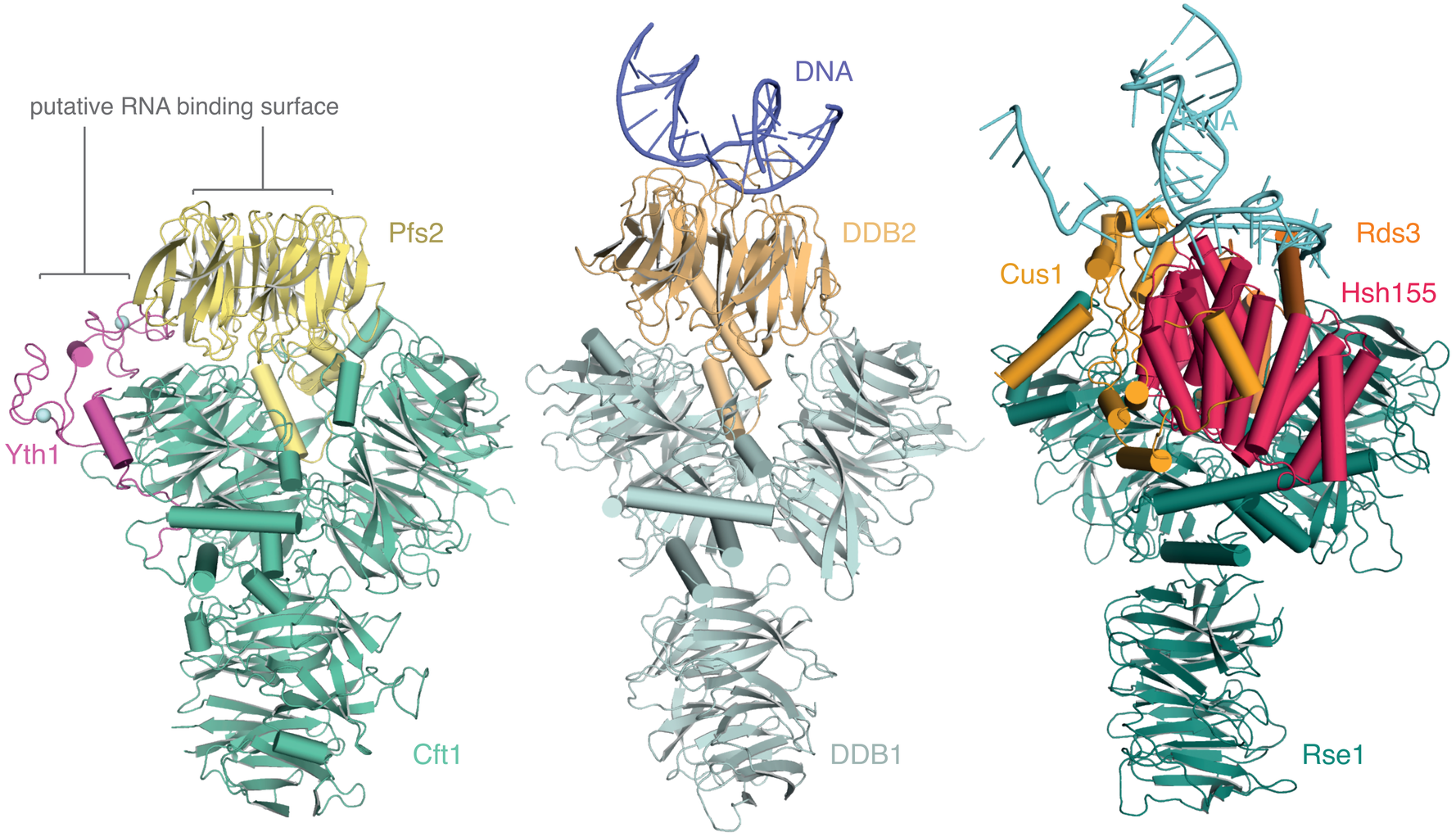
DNA repair
DDB1–DDB2

Scrima et al (2008)

pre-mRNA splicing
SF3b

Yan et al (2016)
Cretu et al (2016)
Plaschka et al (2017)

Similarity to other nucleic acid binding complexes



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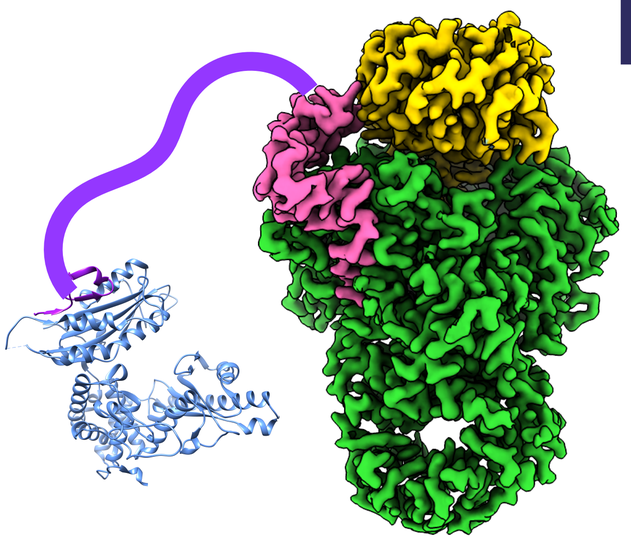
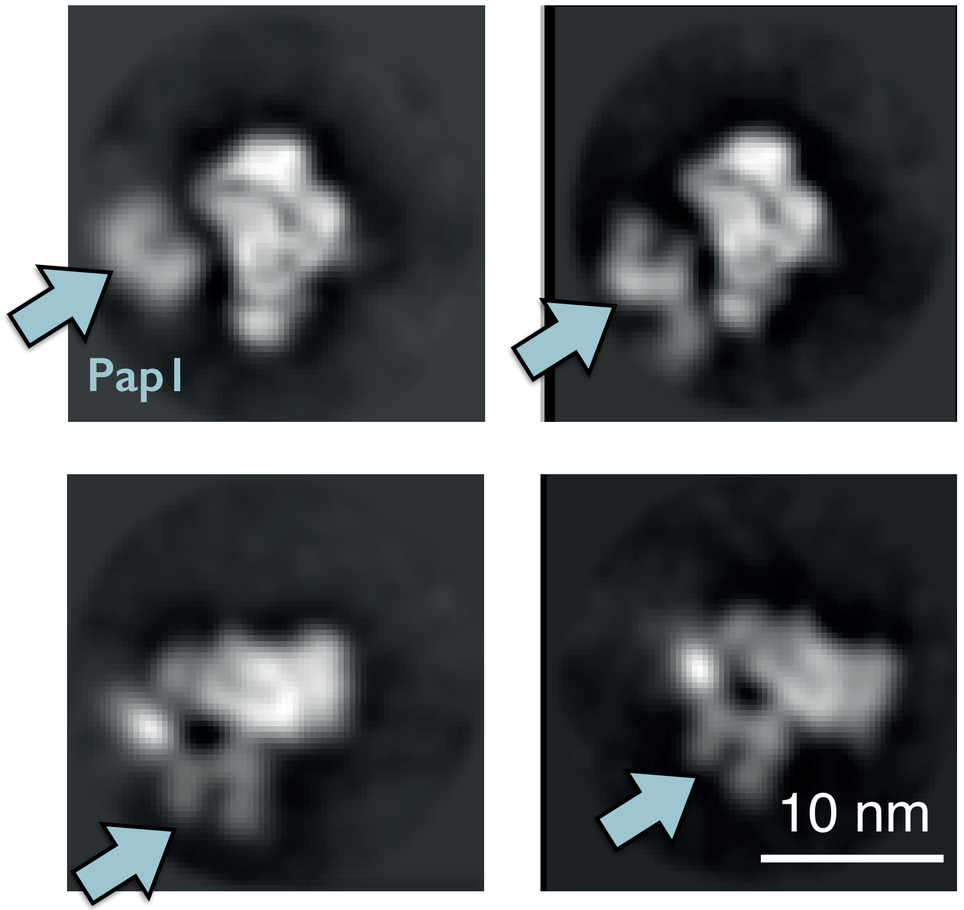
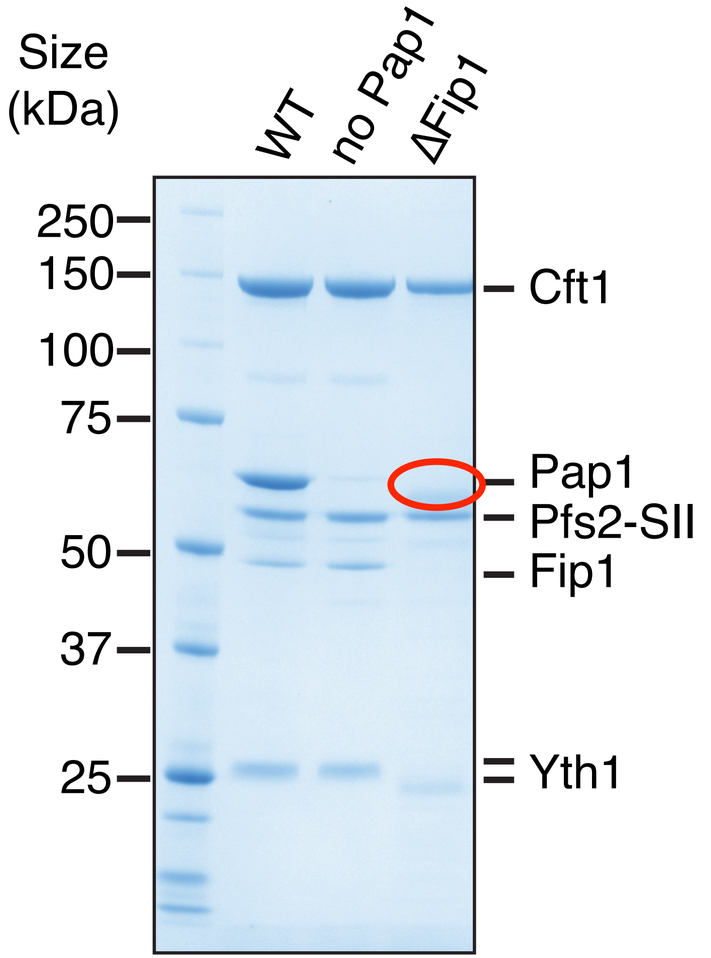
Pap1 is flexibly tethered

Pap1 is flexibly tethered







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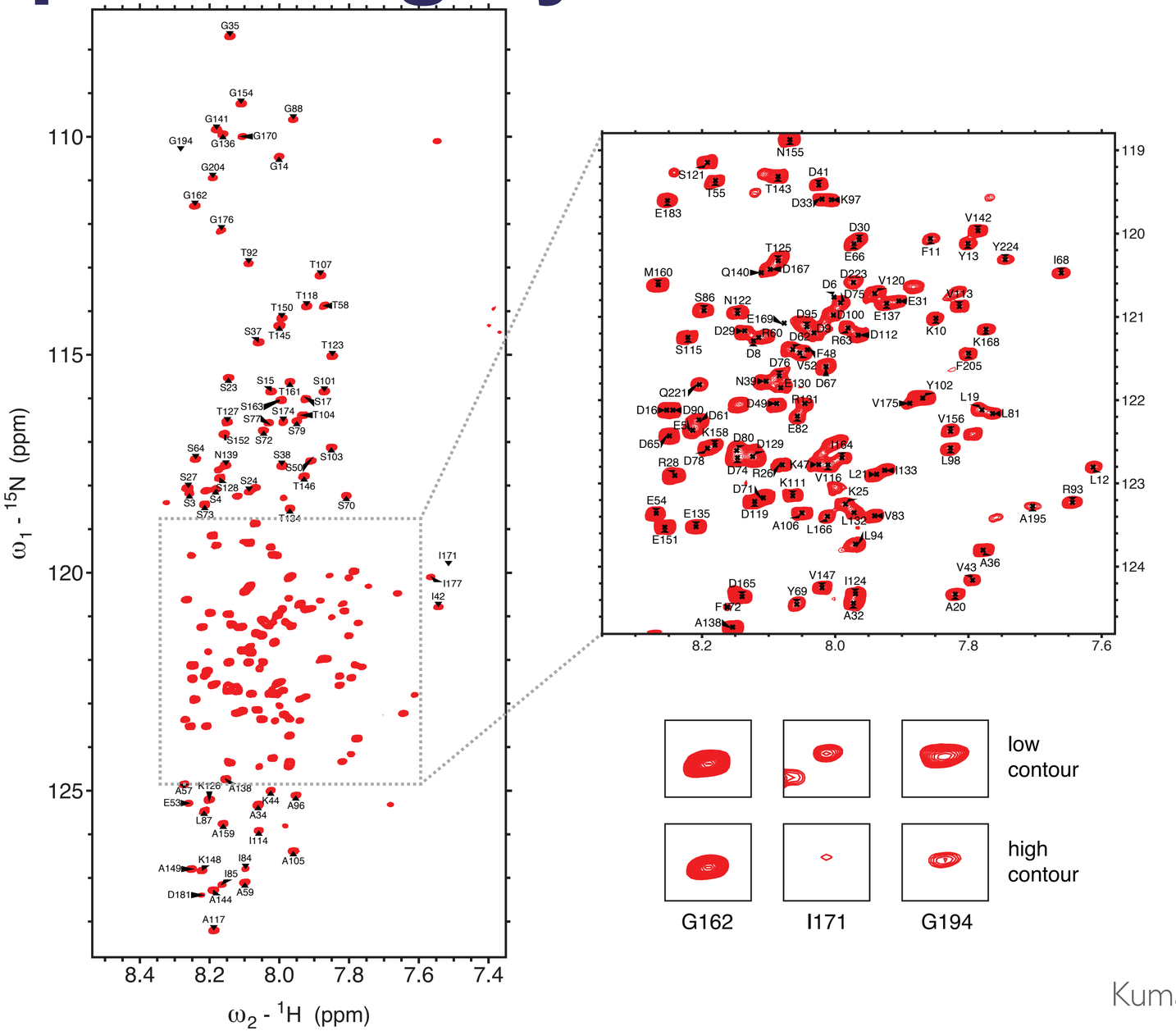
Kumar, Yu et al, 2021 Genes Dev

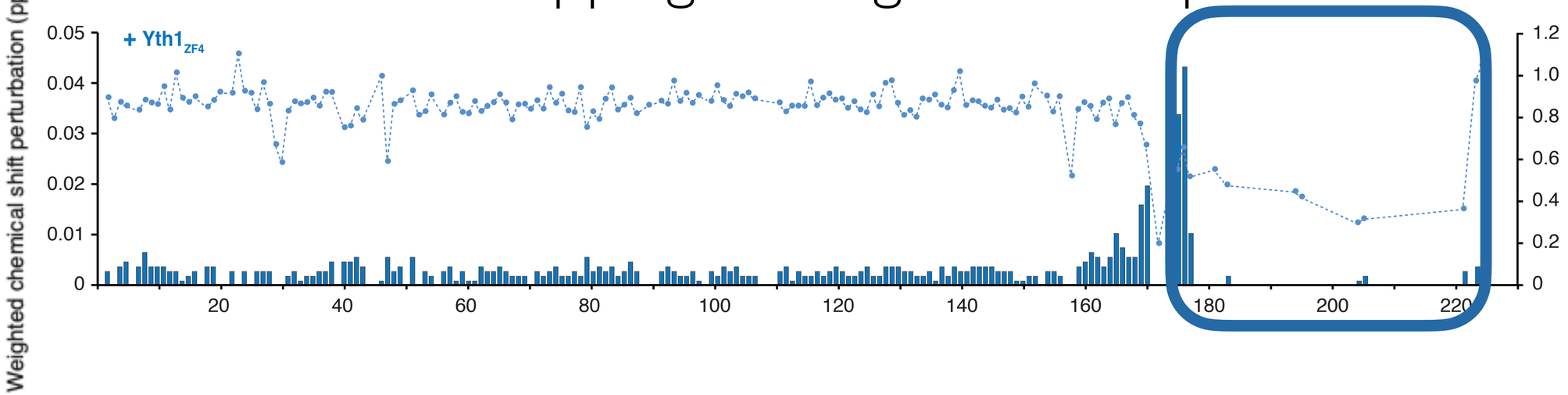
Fip1 is largely disordered



Ananth Kumar,
Conny Yu, Stefan Freund

Kumar, Yu et al, 2021 Genes Dev

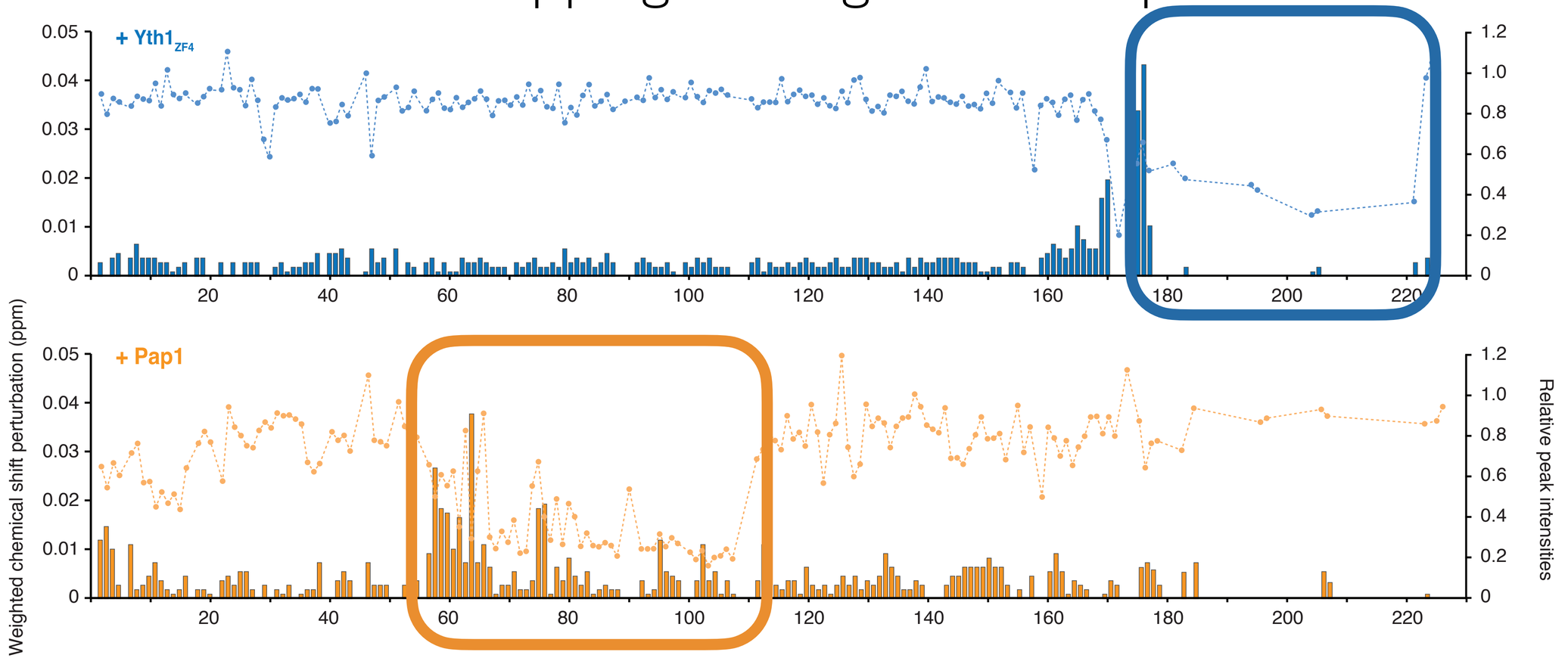
Mapping binding sites on Fip1



+Yth1

Kumar et al, unpublished

Mapping binding sites on Fip1

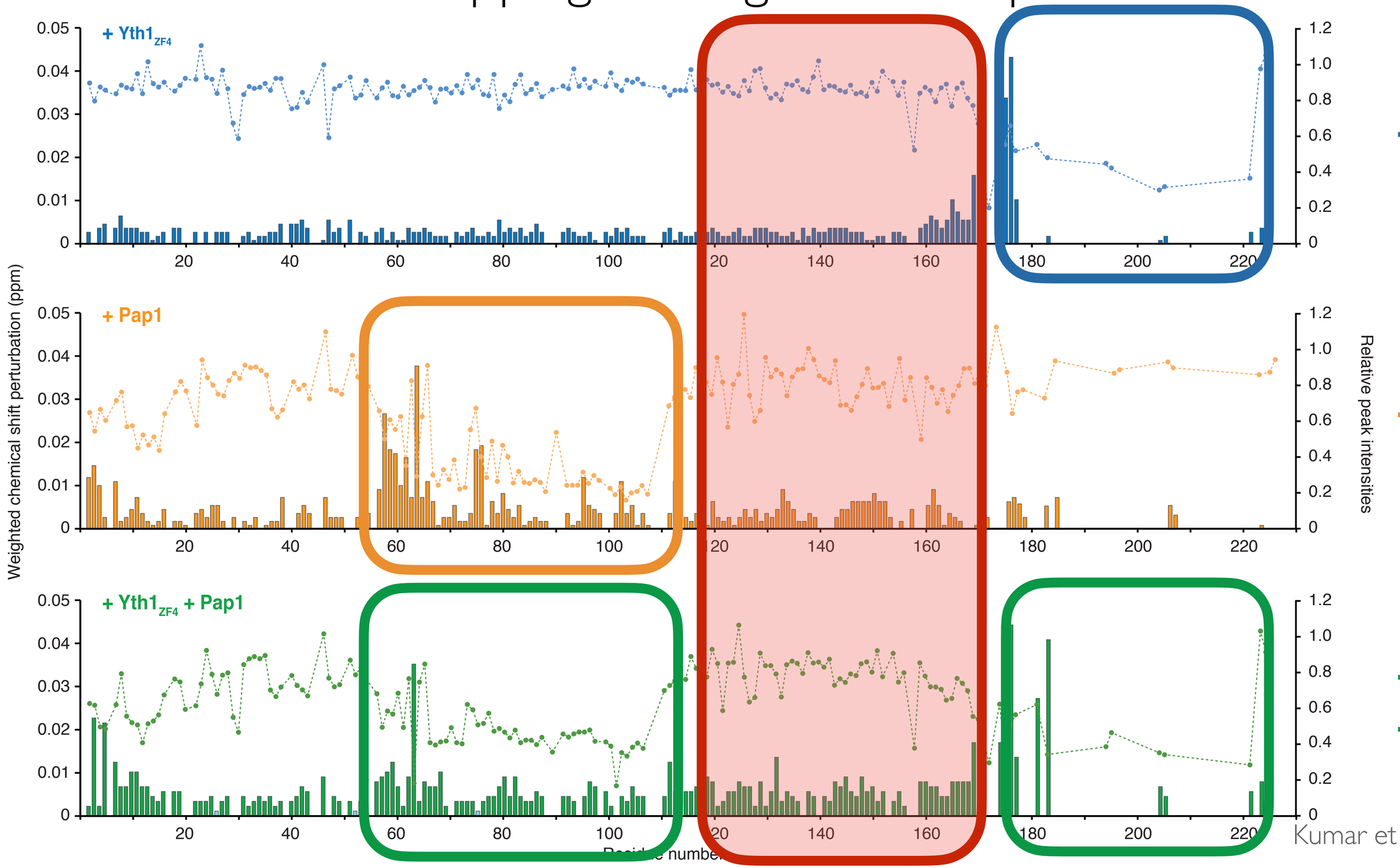


+Yth1

+Pap1

Kumar et al, unpublished

Mapping binding sites on Fip1



+Yth1

+Pap1

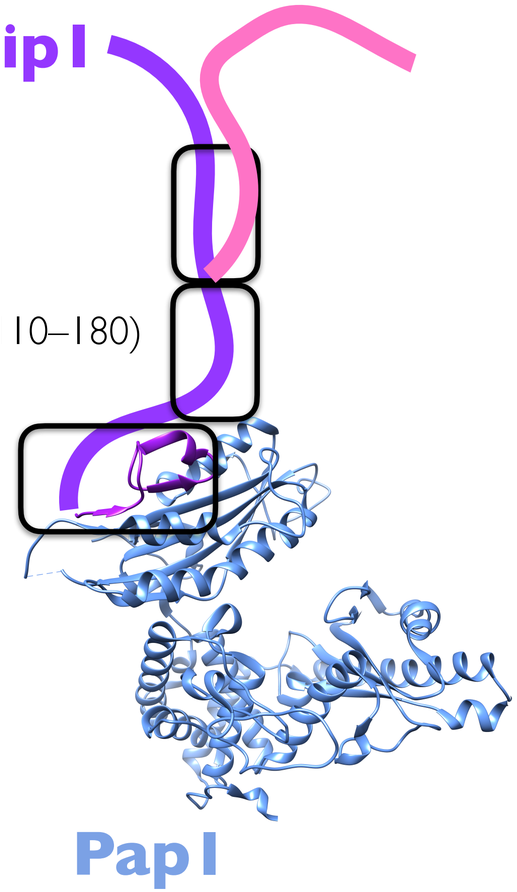
+Yth1
+Pap1

Kumar et al, unpublished

Yth1 binding (180–220)
Hamilton & Tong 2020

Low complexity region (110–180)
Ezeokonkwo et al., 2011

Pap1 binding (60–110)
Meinke et al., 2008



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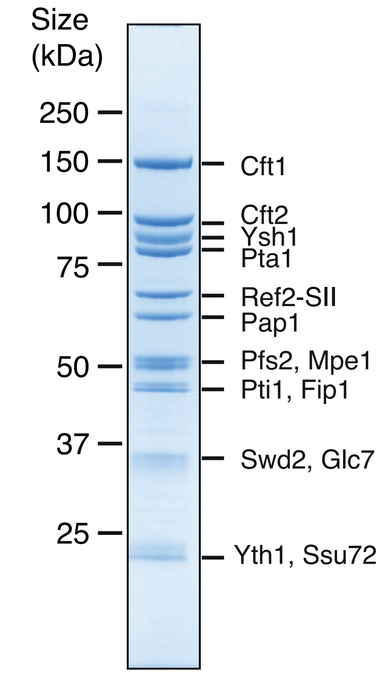
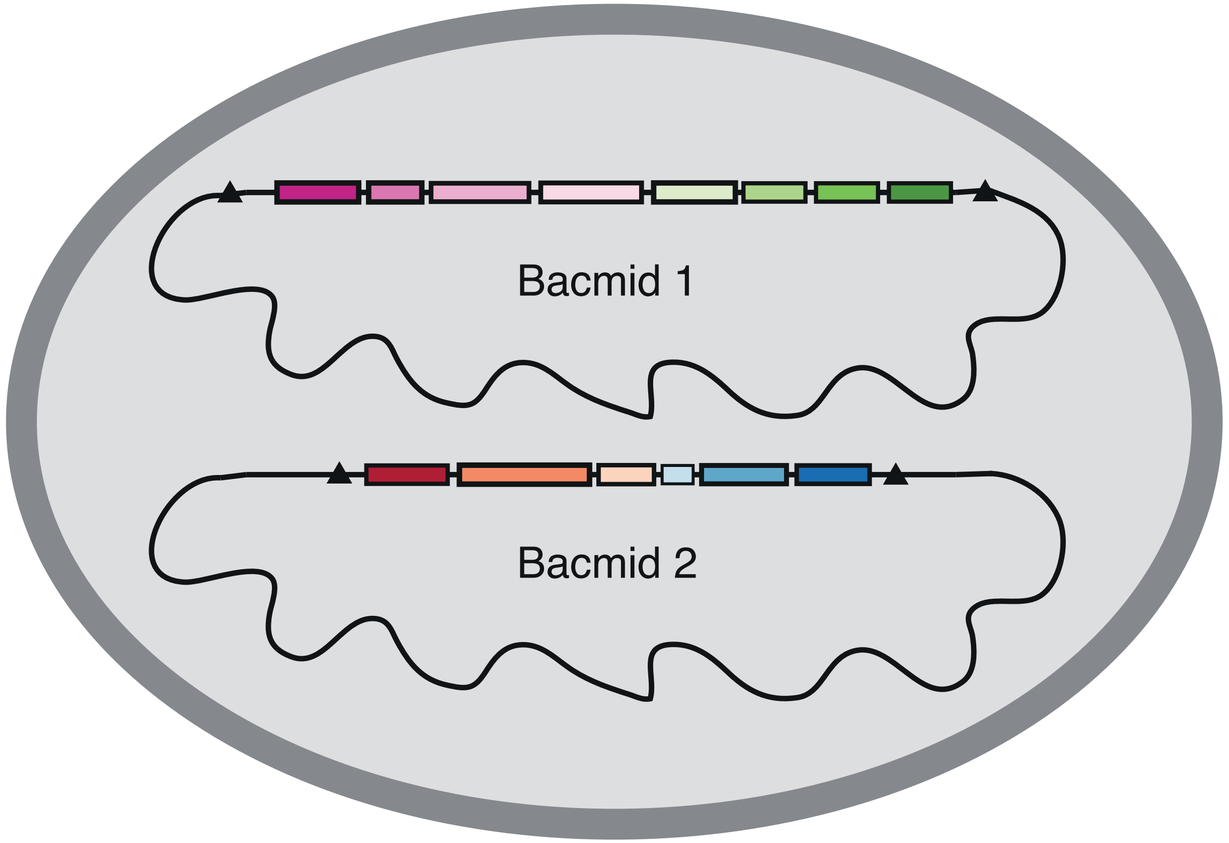
Understanding the dynamics of CPF

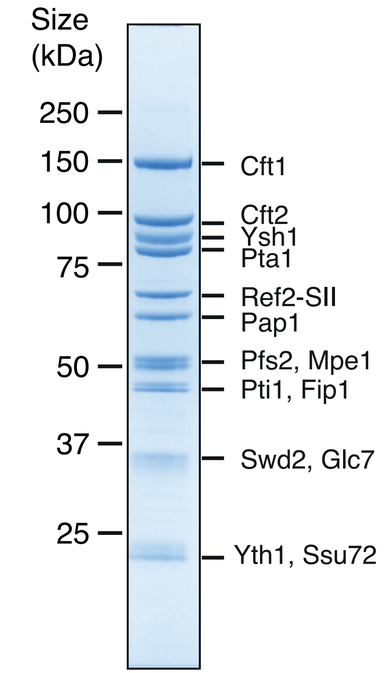


Recombinant CPF

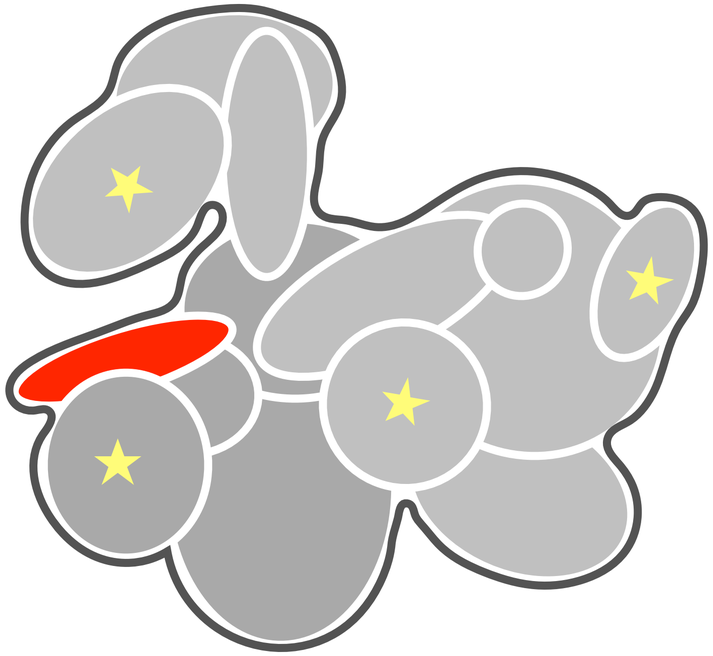


Kumar, Yu et al, 2021 Genes Dev

Understanding the dynamics of CPF

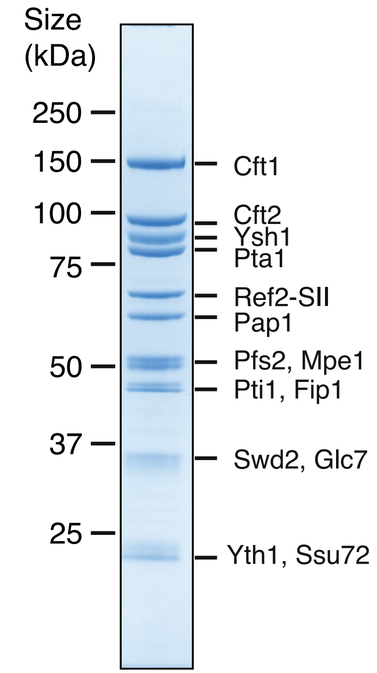


Recombinant CPF

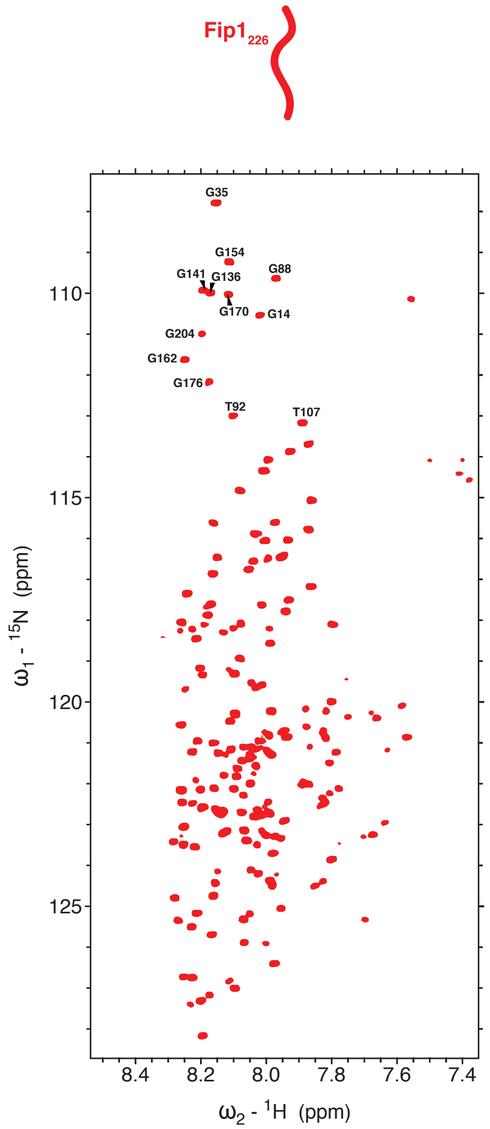


Kumar, Yu et al, 2021 Genes Dev

Understanding the dynamics of CPF

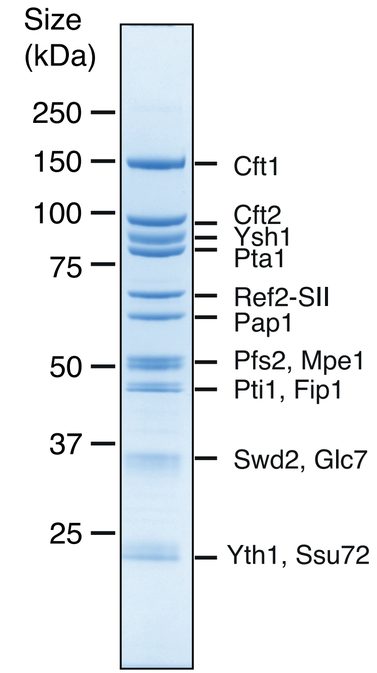


Recombinant CPF



Ananth Kumar, Conny Yu, Stefan Freund
Kumar, Yu et al, 2021 Genes Dev

Understanding the dynamics of CPF



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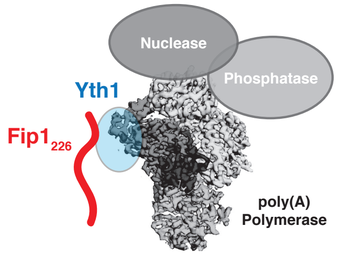

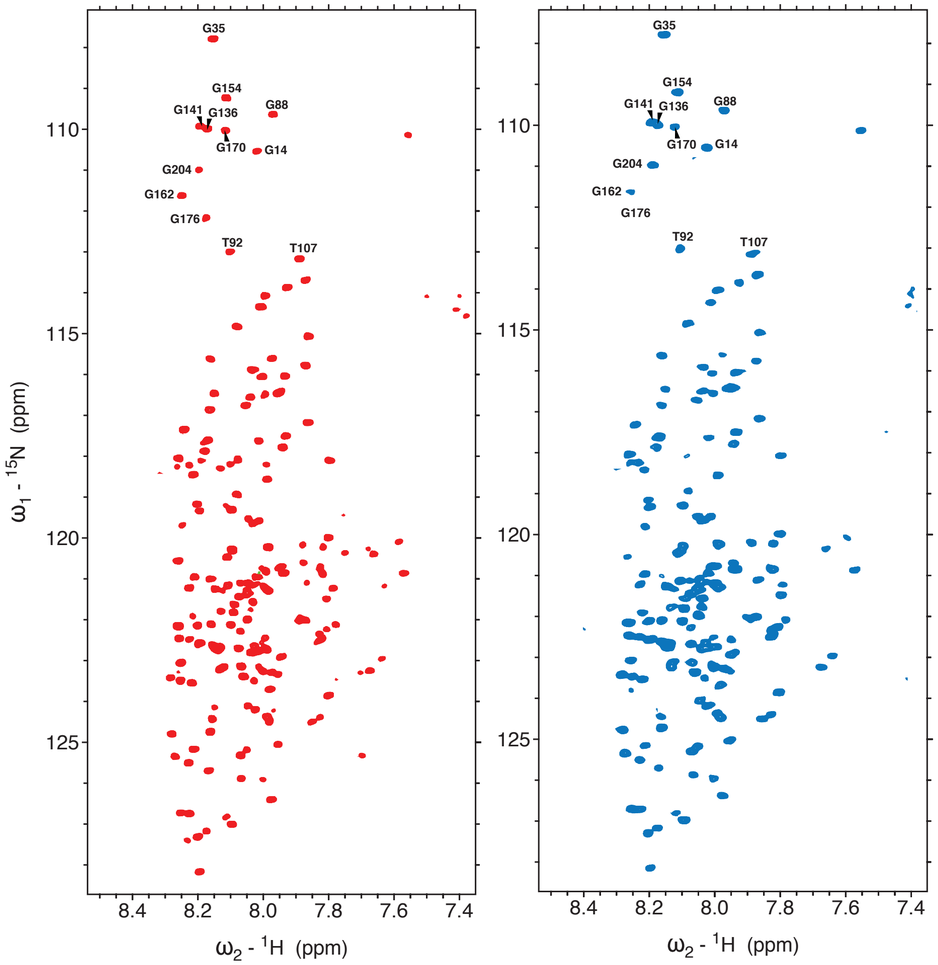

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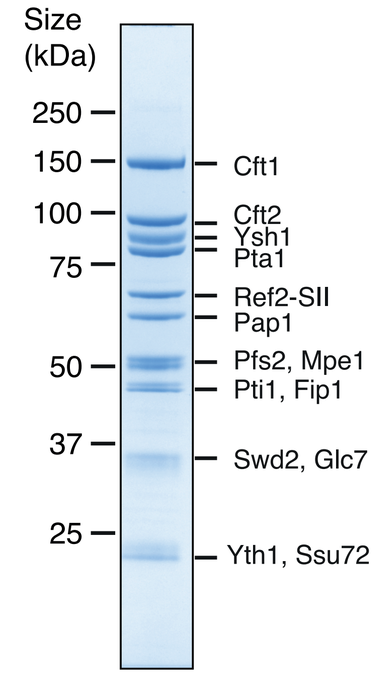
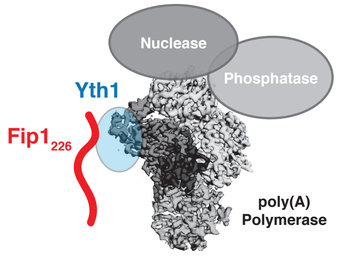
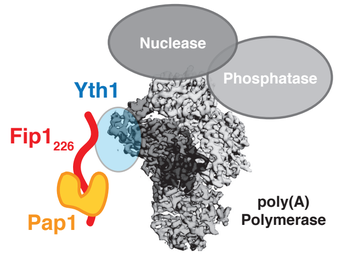
Kumar, Yu et al, 2021 Genes Dev

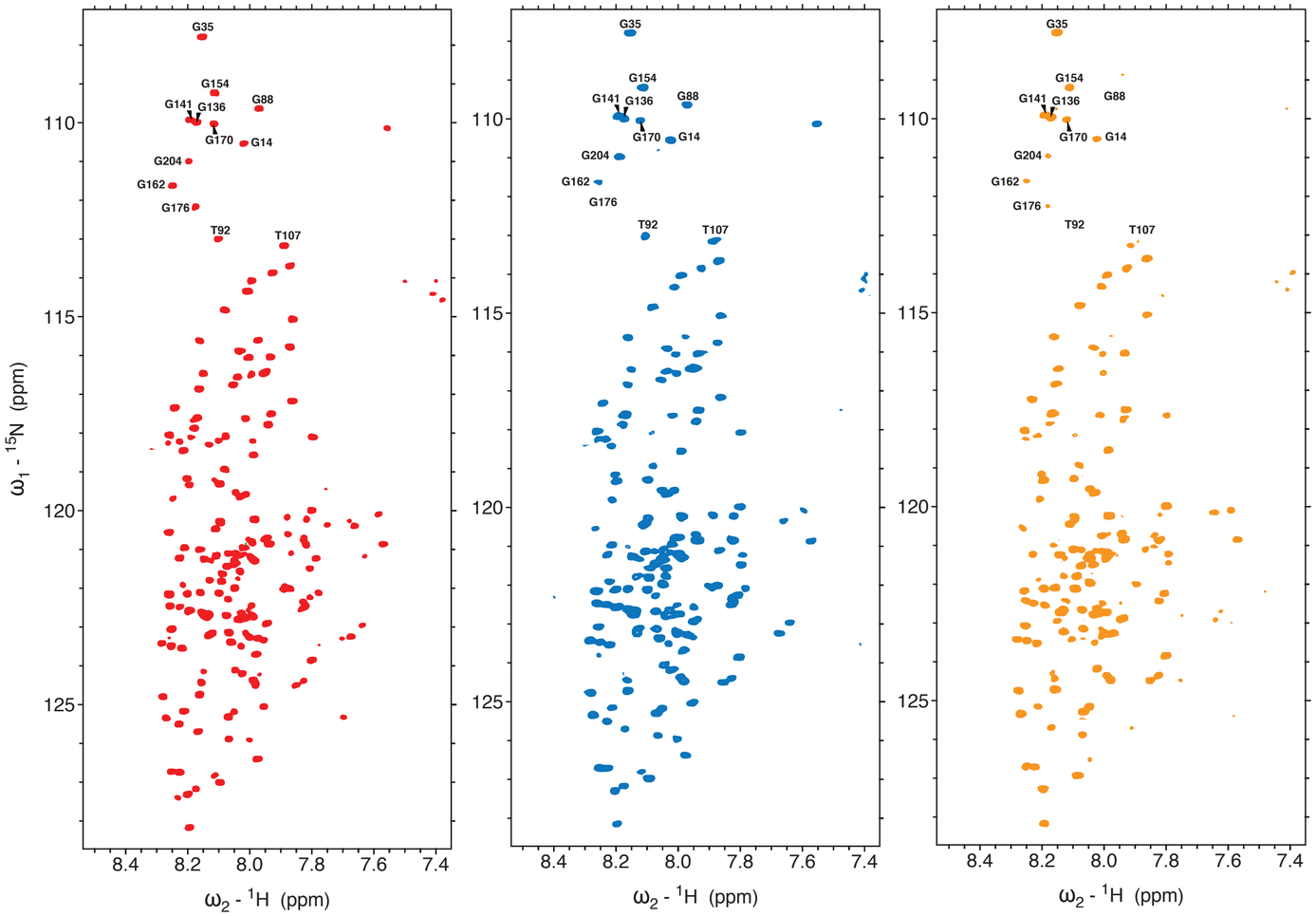
Understanding the dynamics of CPF



Recombinant CPF

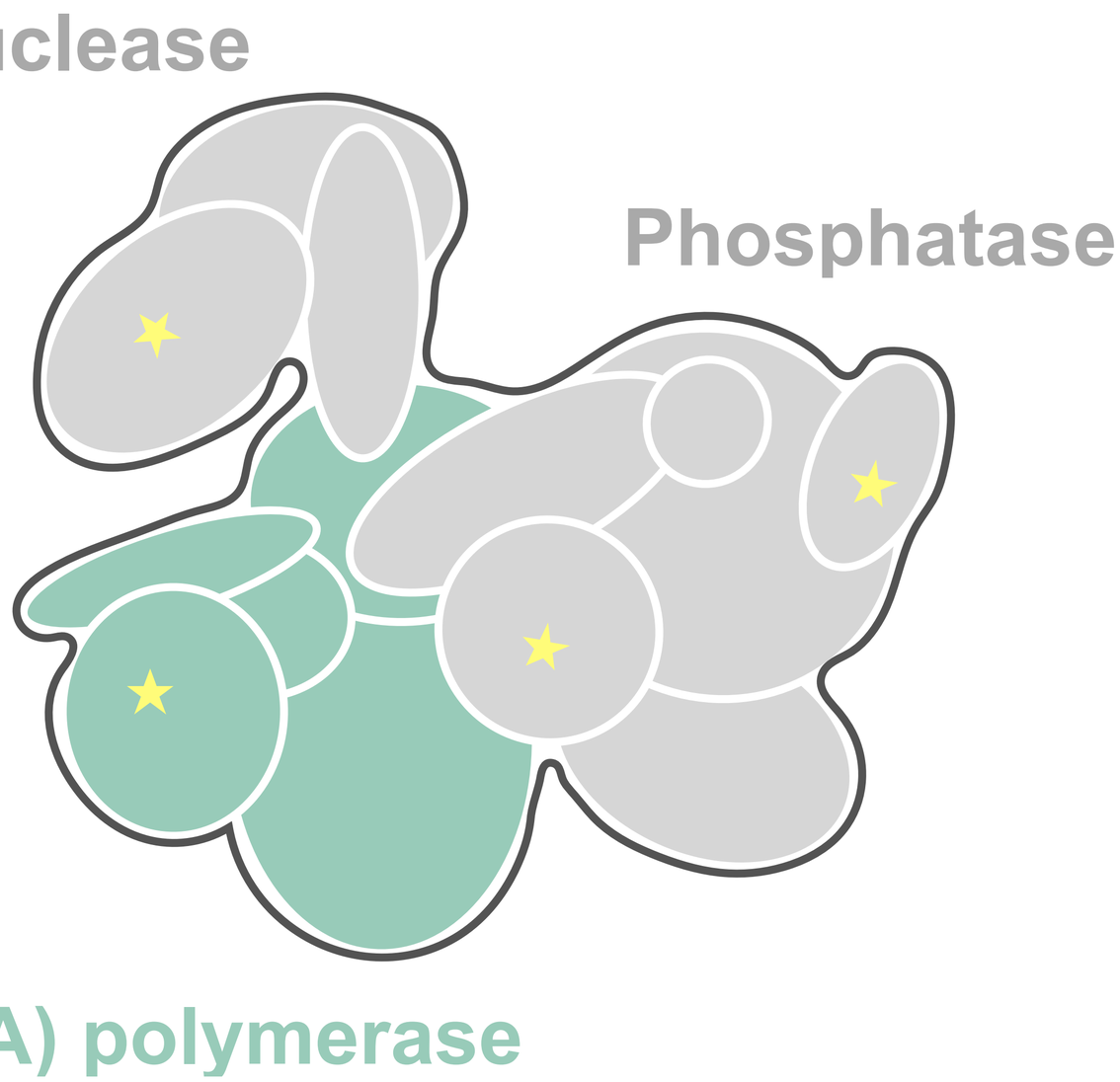




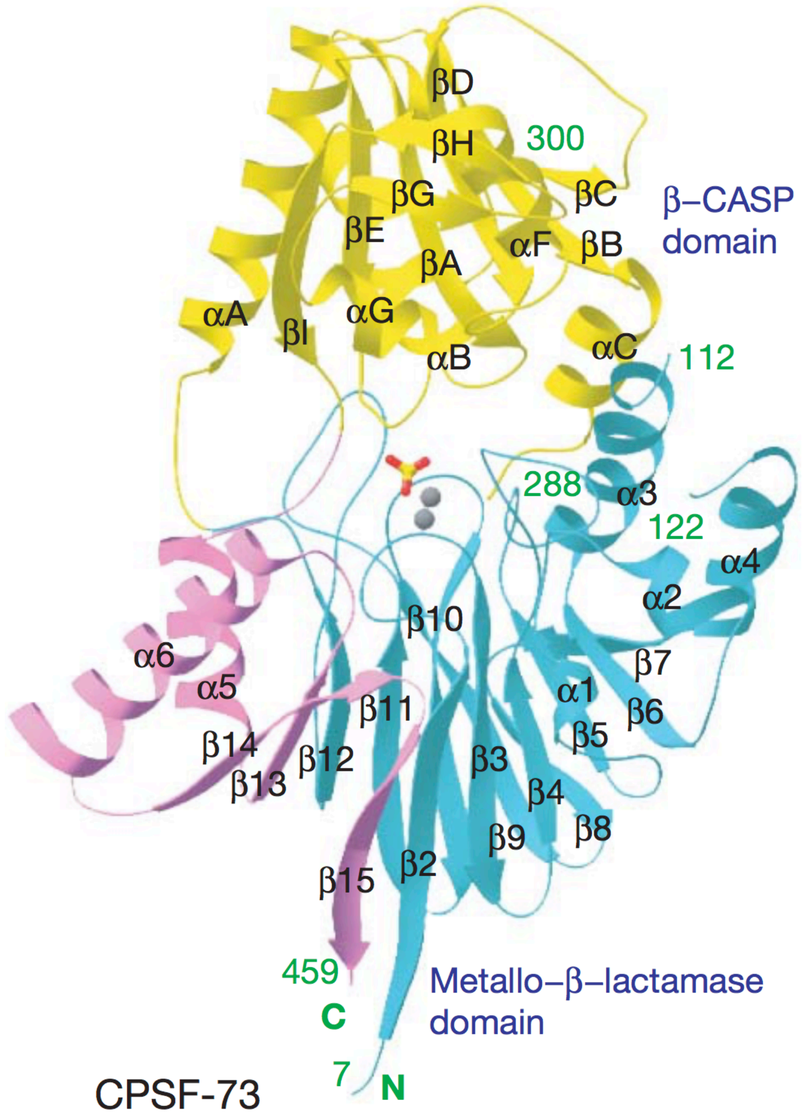


Ananth Kumar, Conny Yu, Stefan Freund
Kumar, Yu et al, 2021 Genes Dev

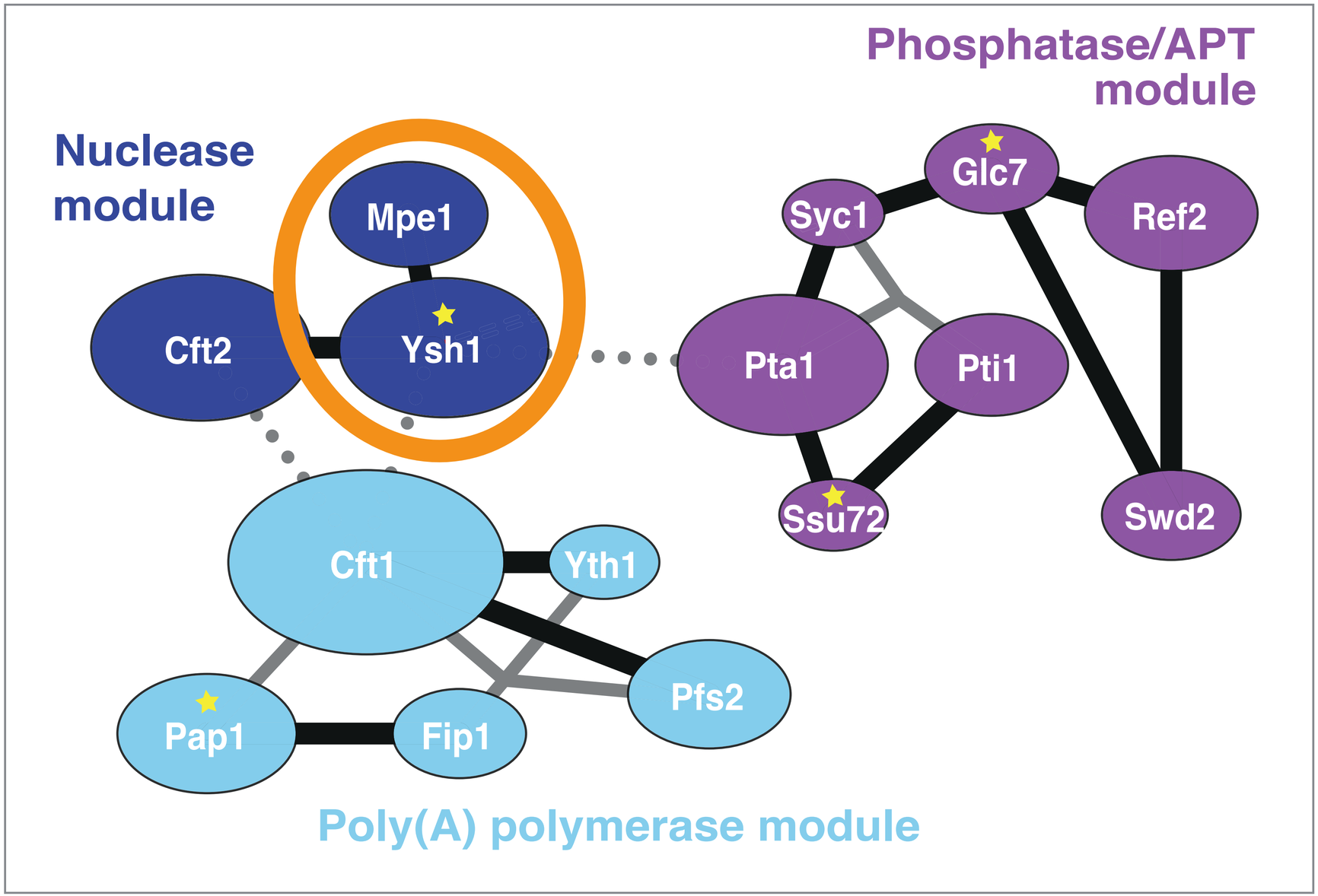
How is the nuclease activated?

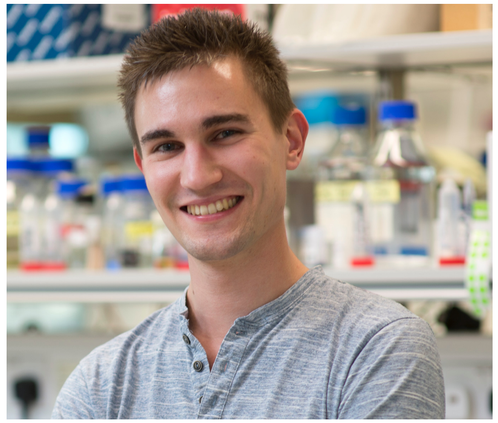


CPF nuclease



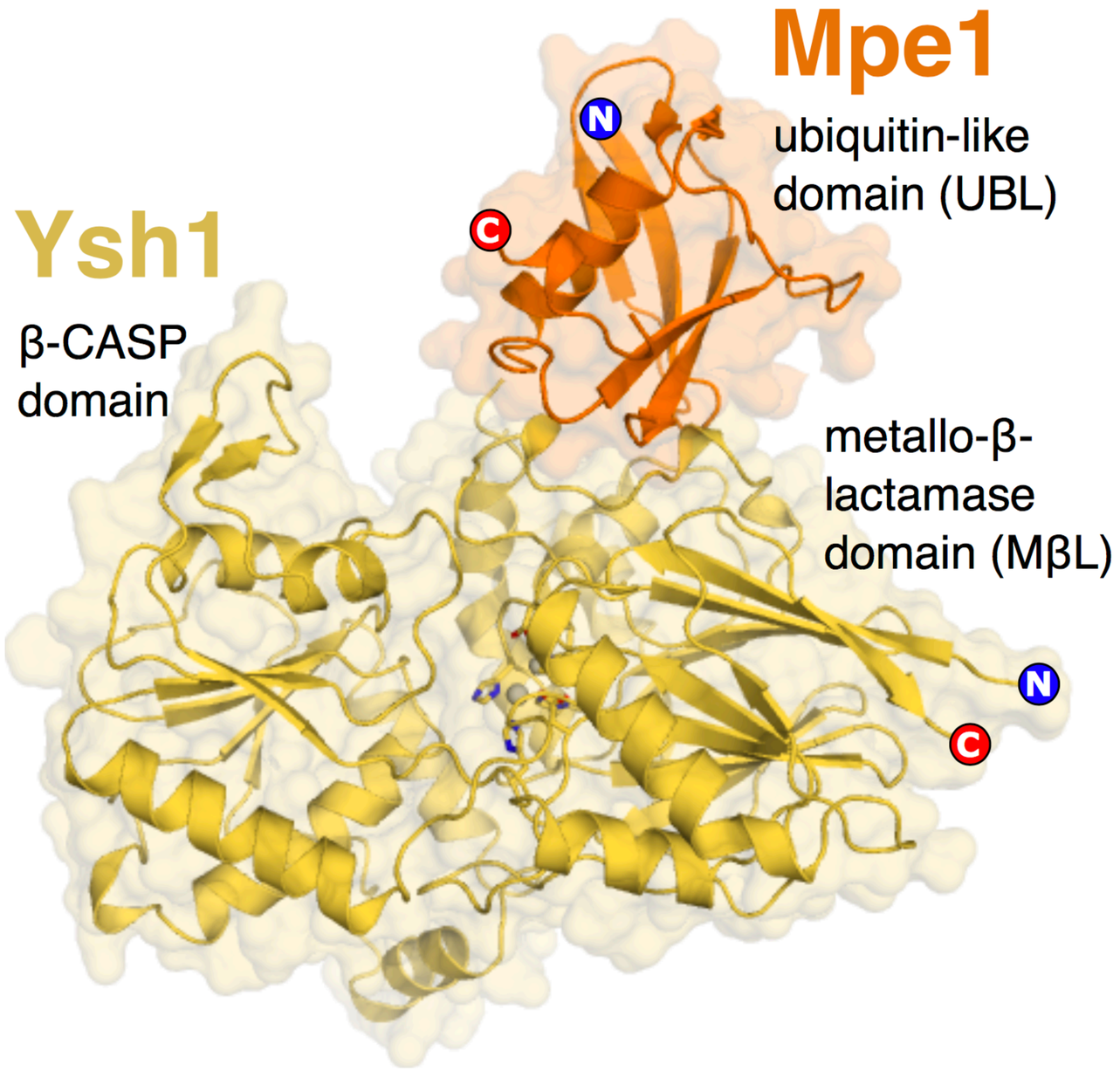
Mandel et al (2006) Tong lab

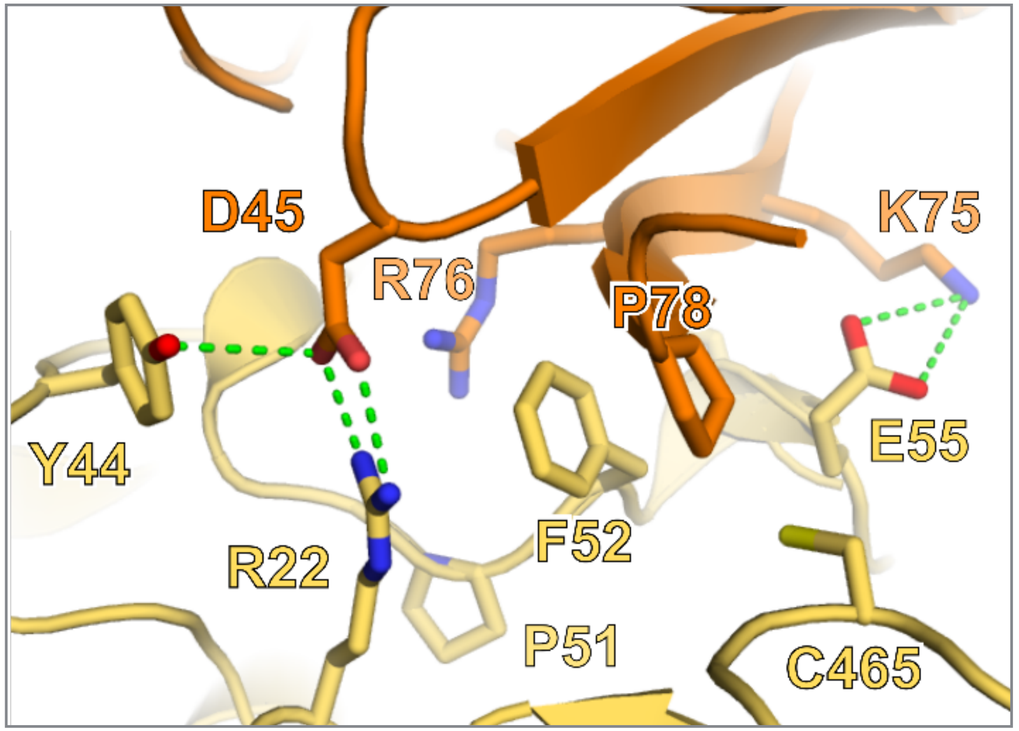

Phosphatase/APT module
Nuclease module
Mpe1
Cft2
Ysh1
Glc7
Syc1
Ref2
Pta1
Pti1
Ssu72
Swd2
Cft1
Yth1
Pfs2
Pap1
Fip1
Poly(A) polymerase module



Chris Hill

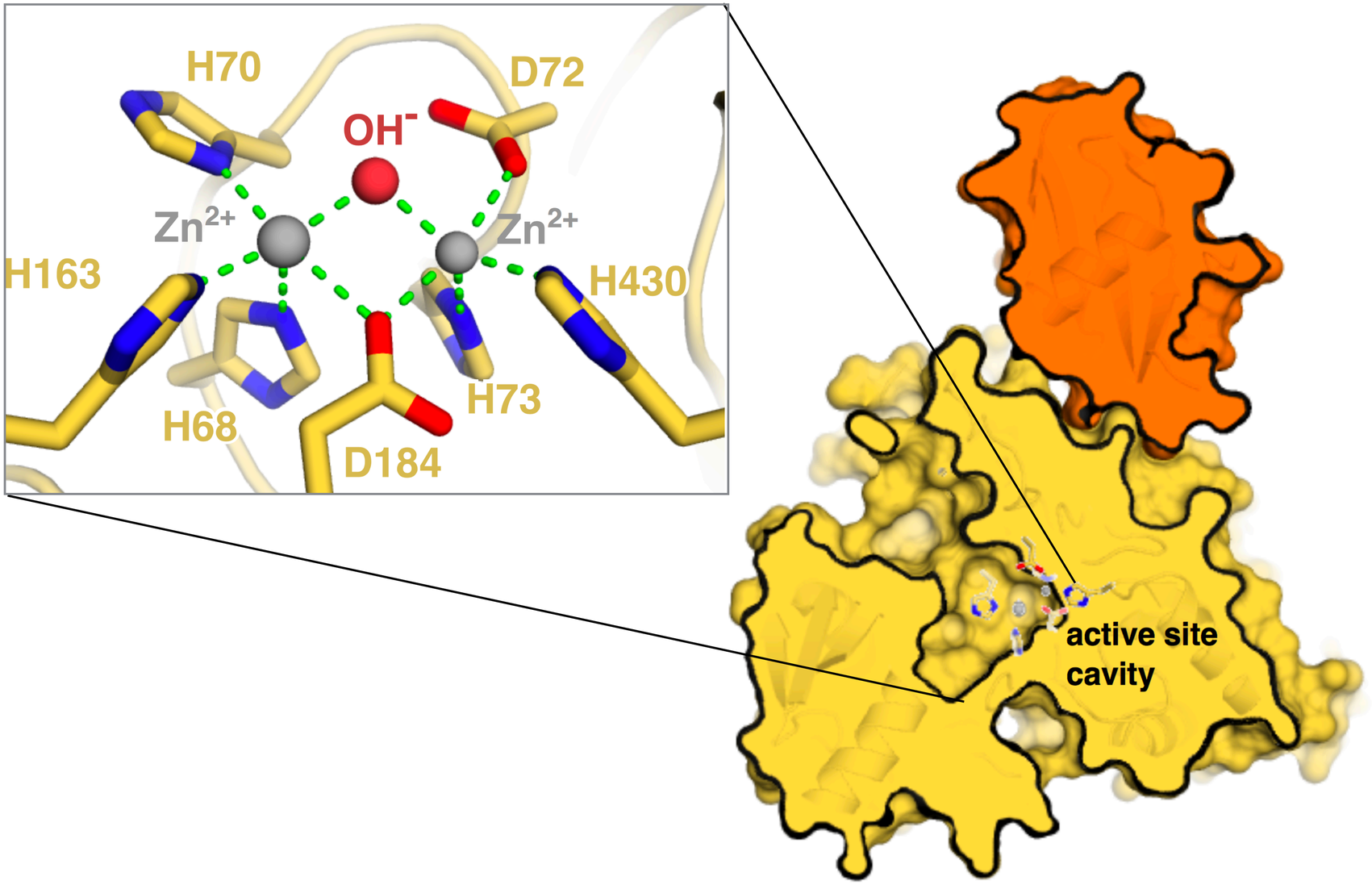
X-ray crystal and cryoEM structure of Ysh1-Mpe1 complex





PDB 6I1D, EMD-0325
Chris Hill, Vytaute Borekaite
Hill et al., Mol Cell 2019

The active site is located at the end of a long tunnel



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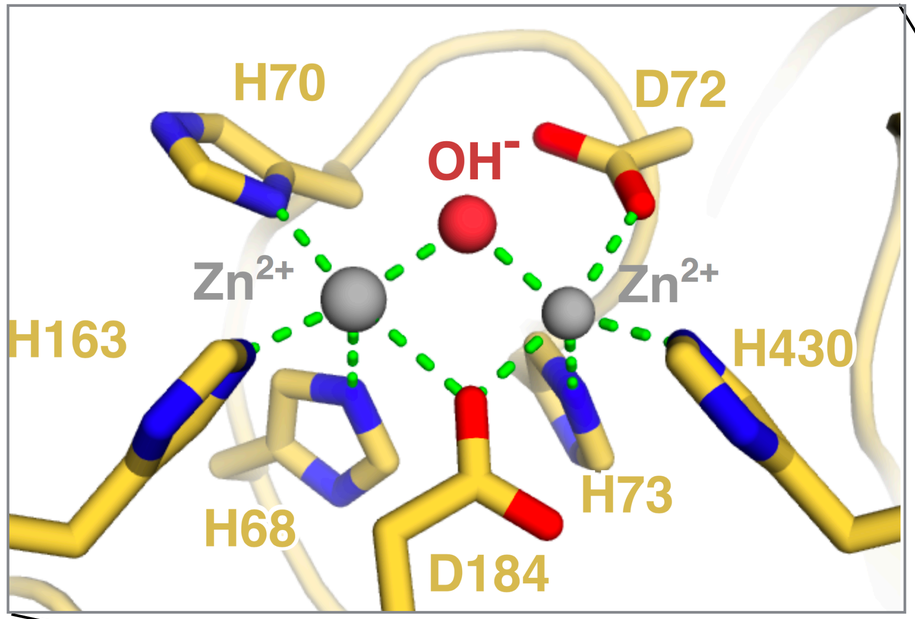
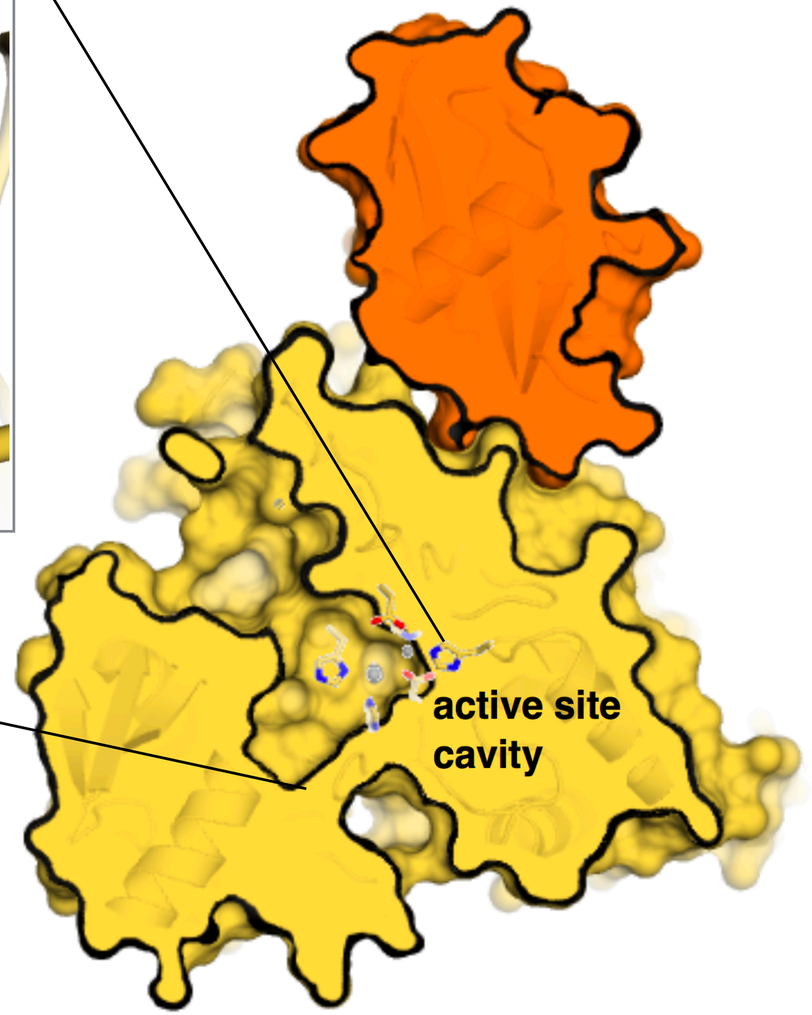
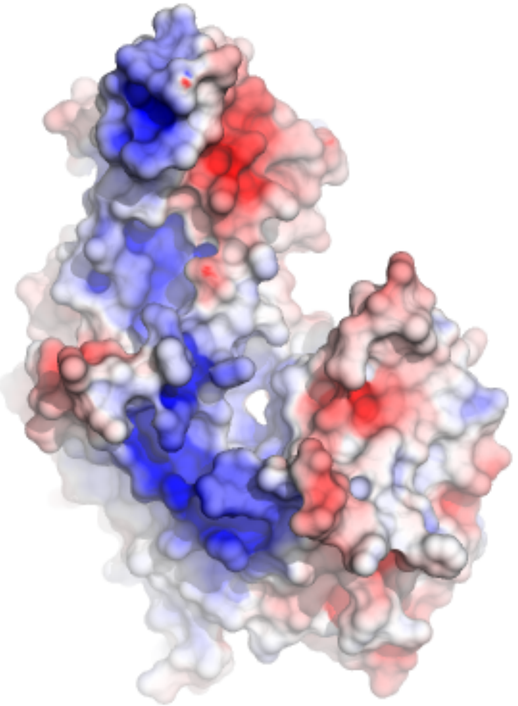
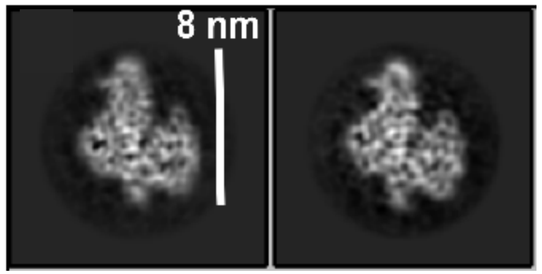


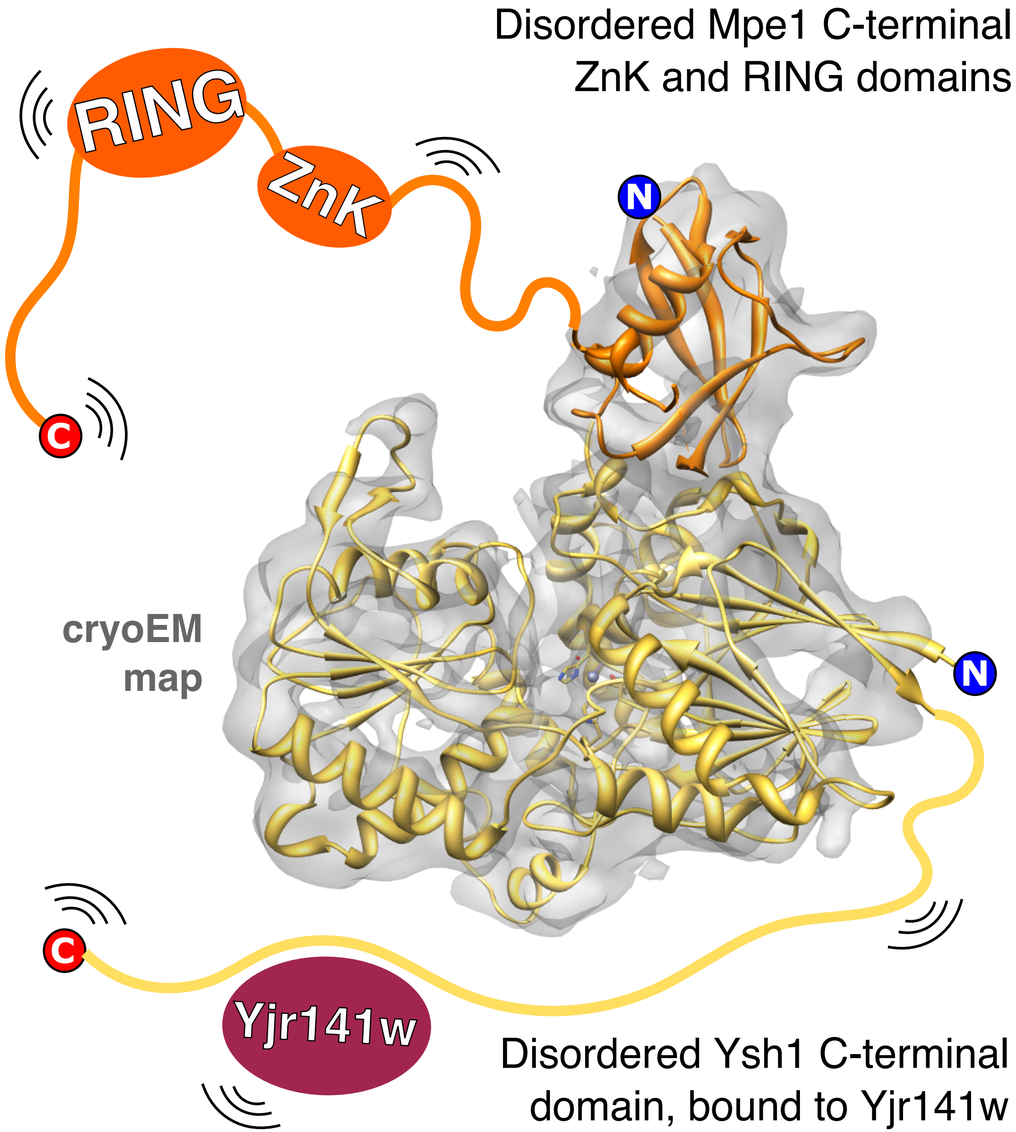




PDB 6I1D, EMD-0325
Chris Hill, Vytaute Borekaite
Hill et al., Mol Cell 2019

Cryo-EM of Ysh1-Mpe1-Ipa1 complex

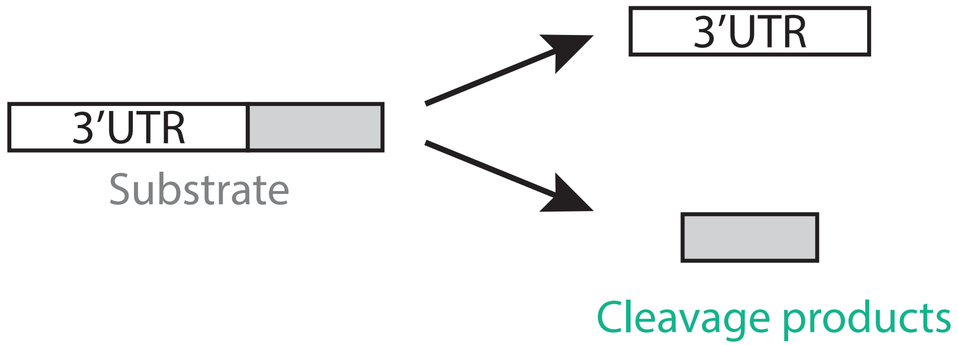


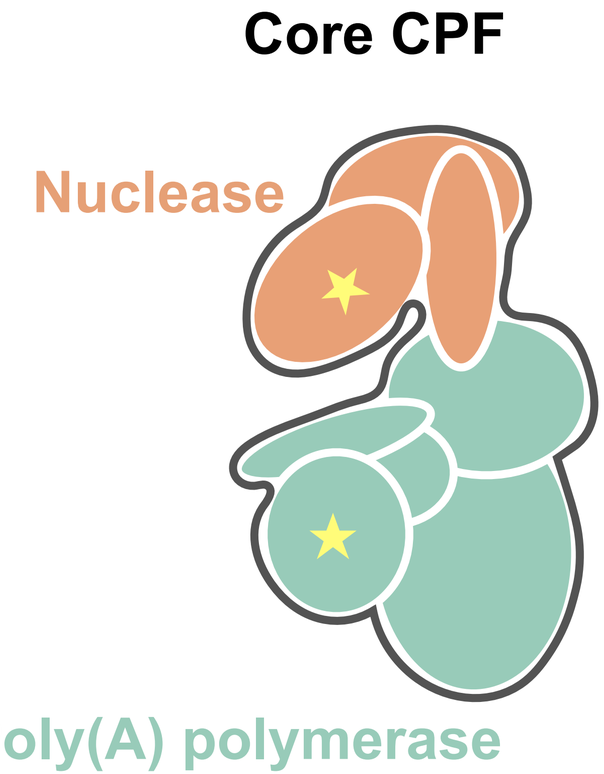


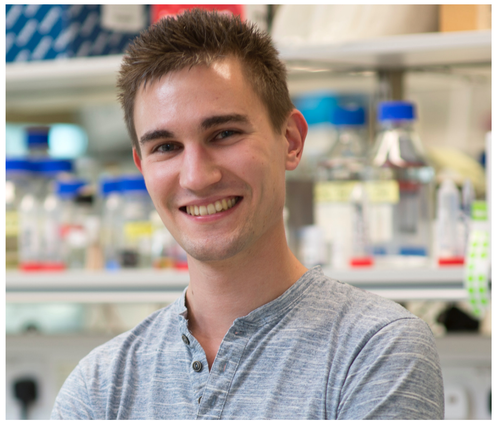
57 kDa

Hill et al., Mol Cell 2019

Reconstitution of cleavage activity by the Ysh1 nuclease





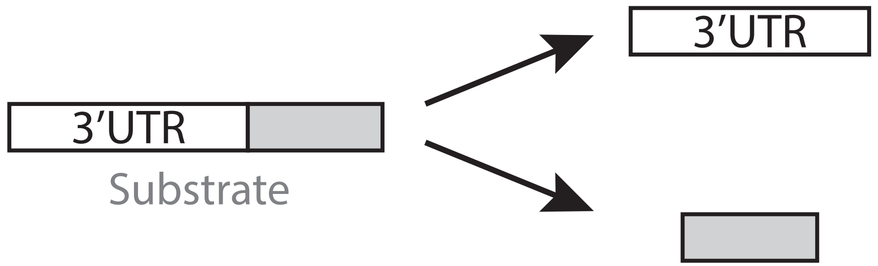


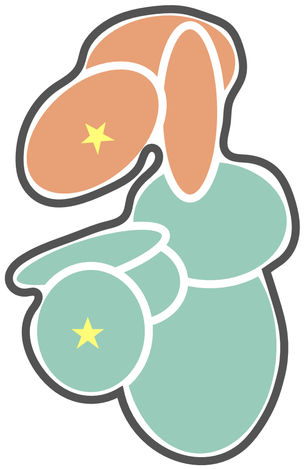
Chris Hill

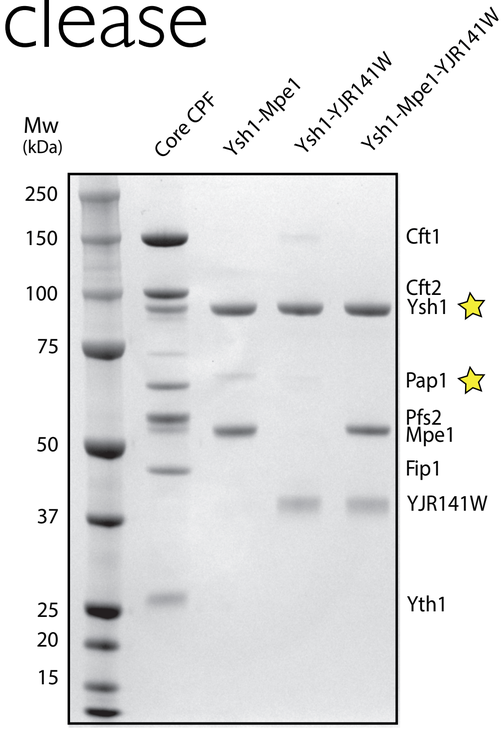
Accessory factors CF IA and CF IB also proposed to play a role in 3' end processing

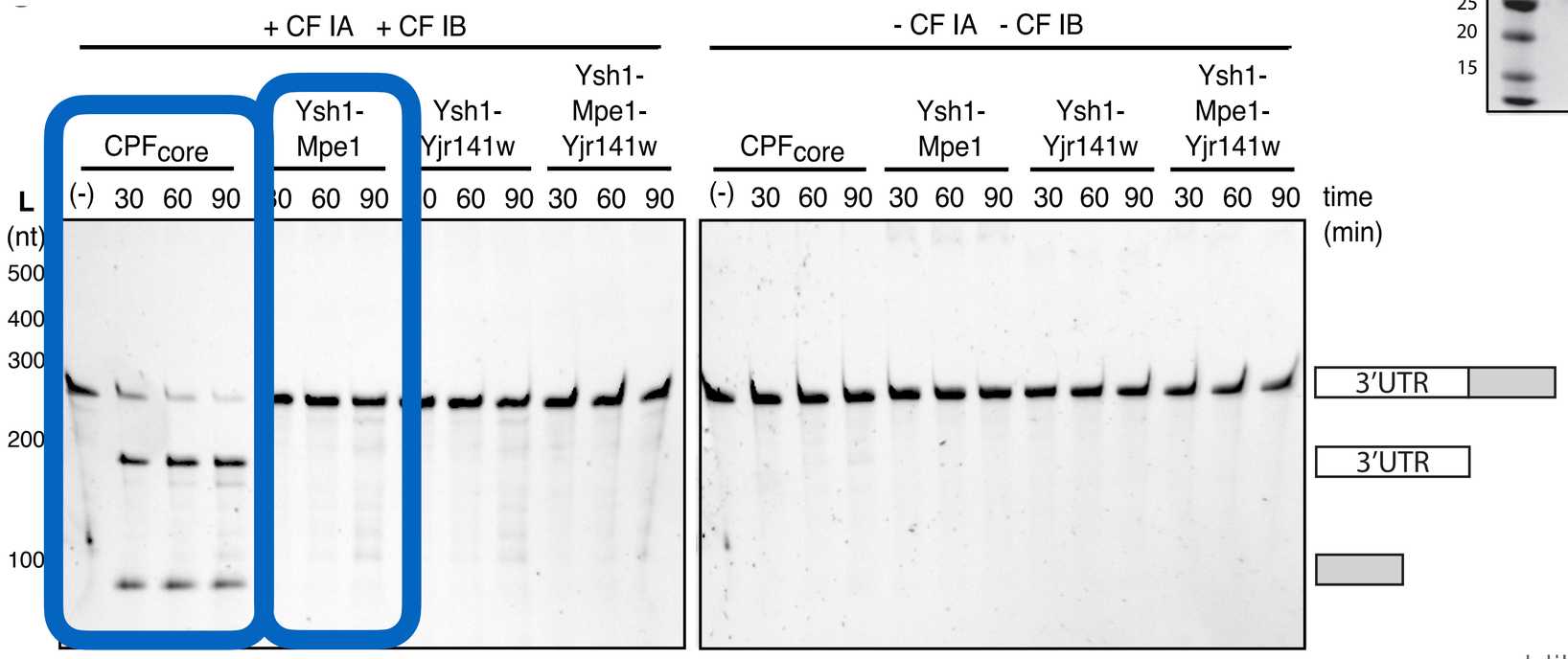
Hill et al., Mol Cell 2019

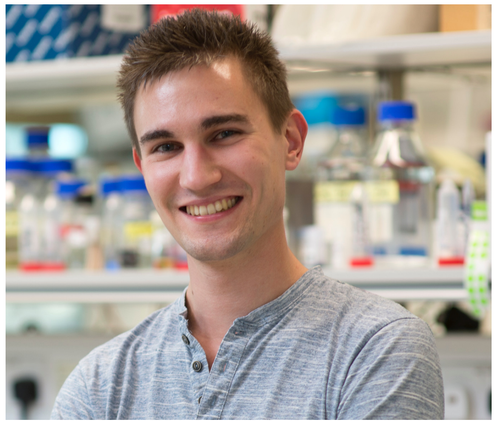
Reconstitution of cleavage activity by the Ysh1 nuclease







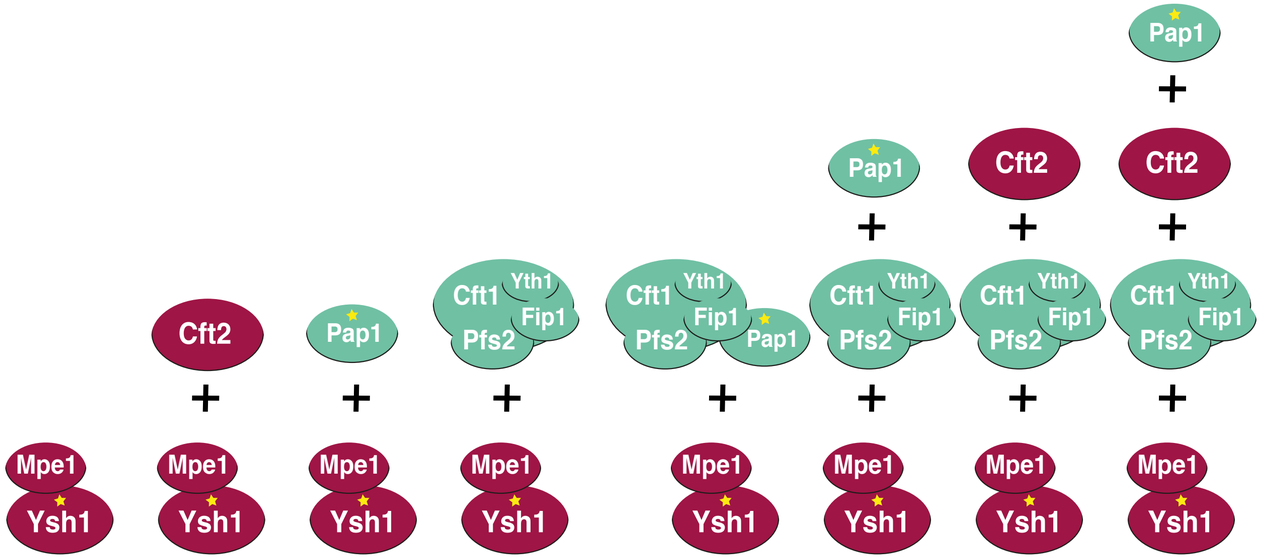




Chris Hill

Hill et al., Mol Cell 2019

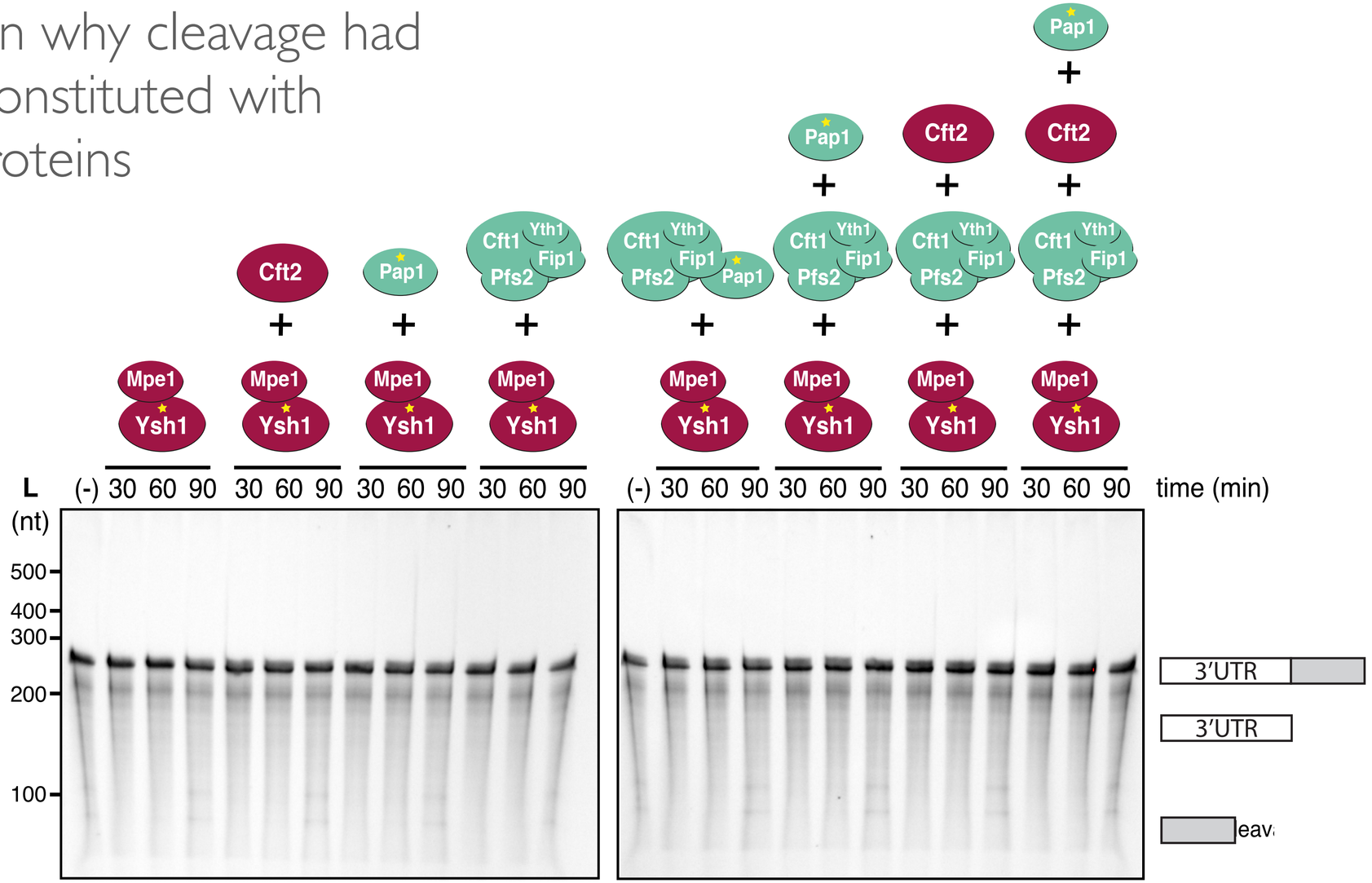
Reconstitution of cleavage activity



Hill et al., Mol Cell 2019

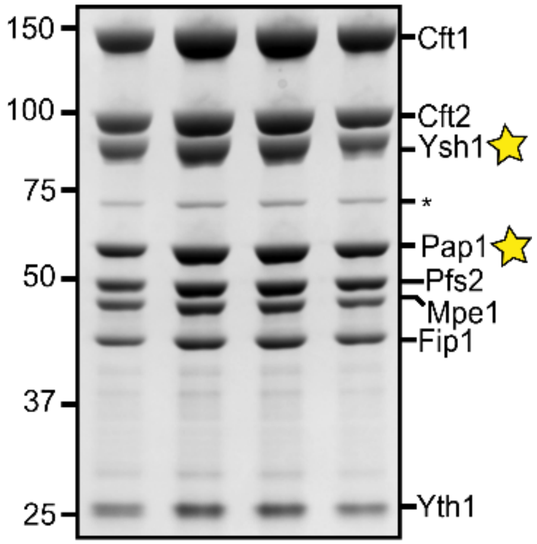
Reconstitution of cleavage activity

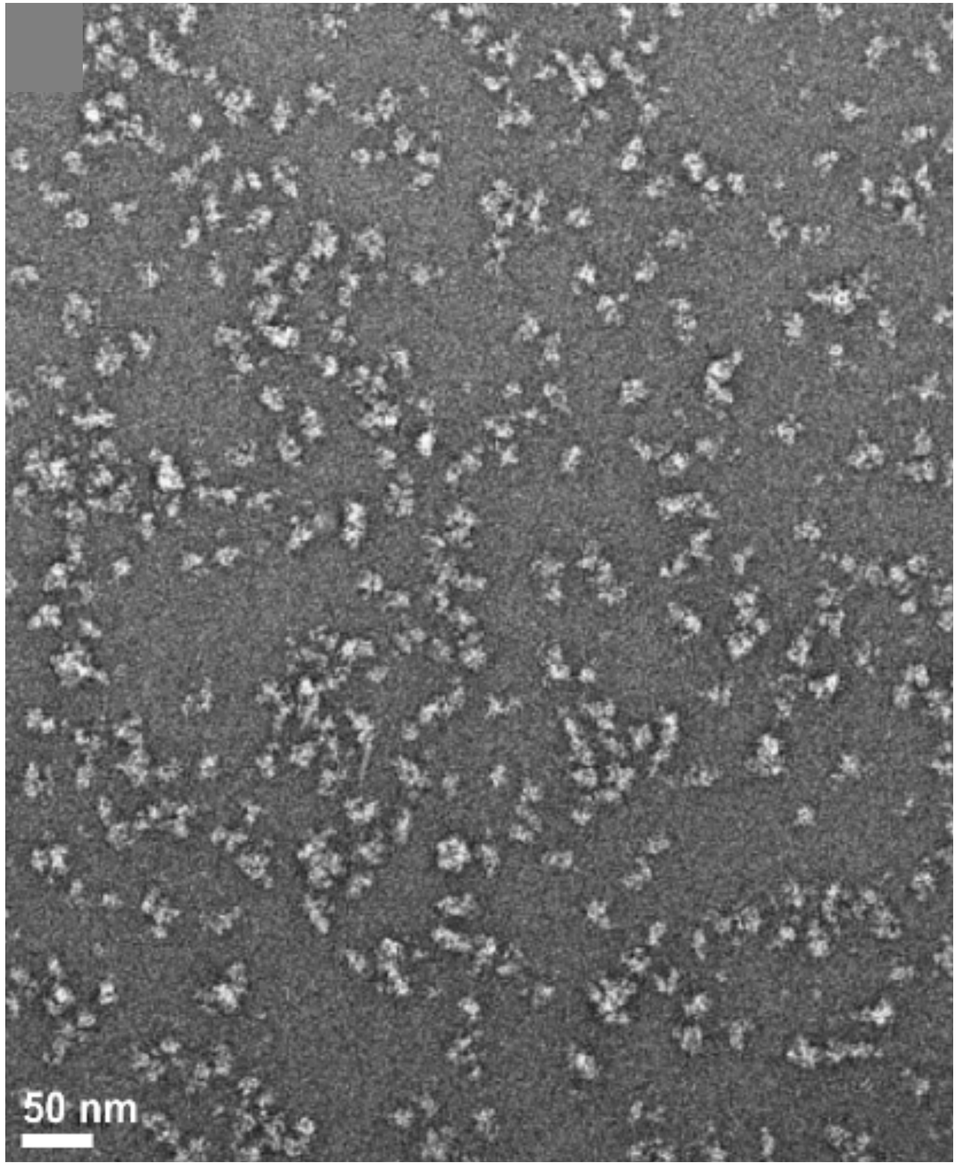
This may explain why cleavage had never been reconstituted with recombinant proteins

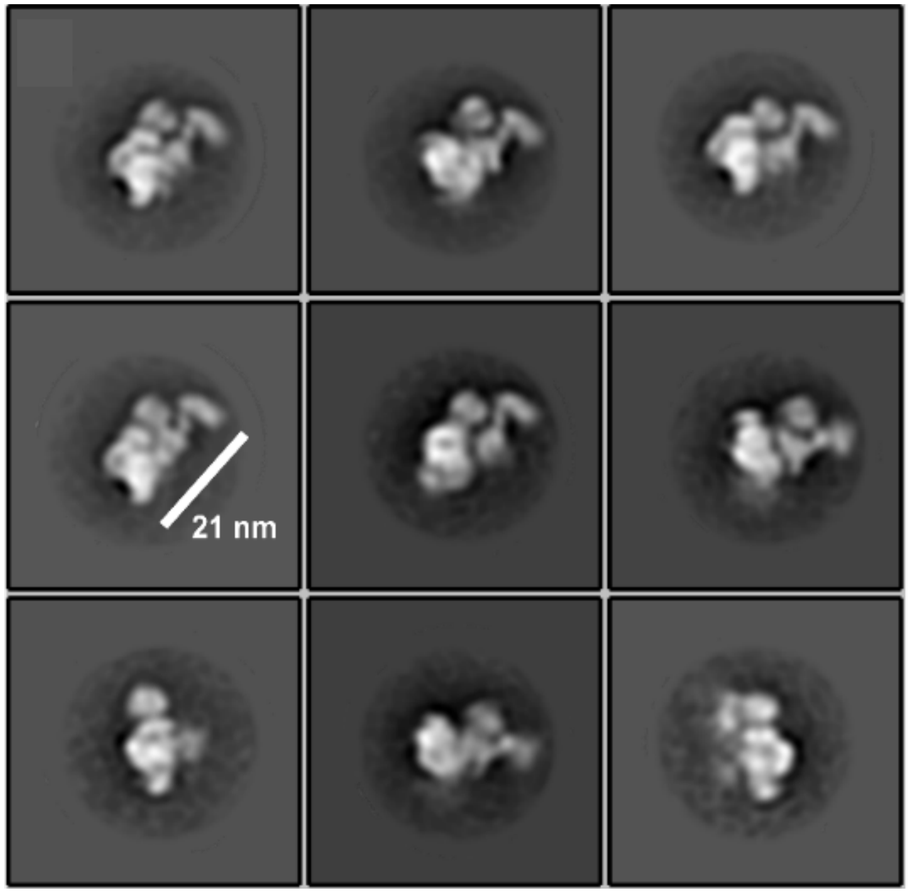


Hill et al., Mol Cell 2019

Negative Stain EM of Core CPF

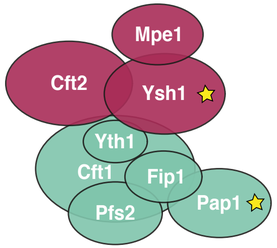


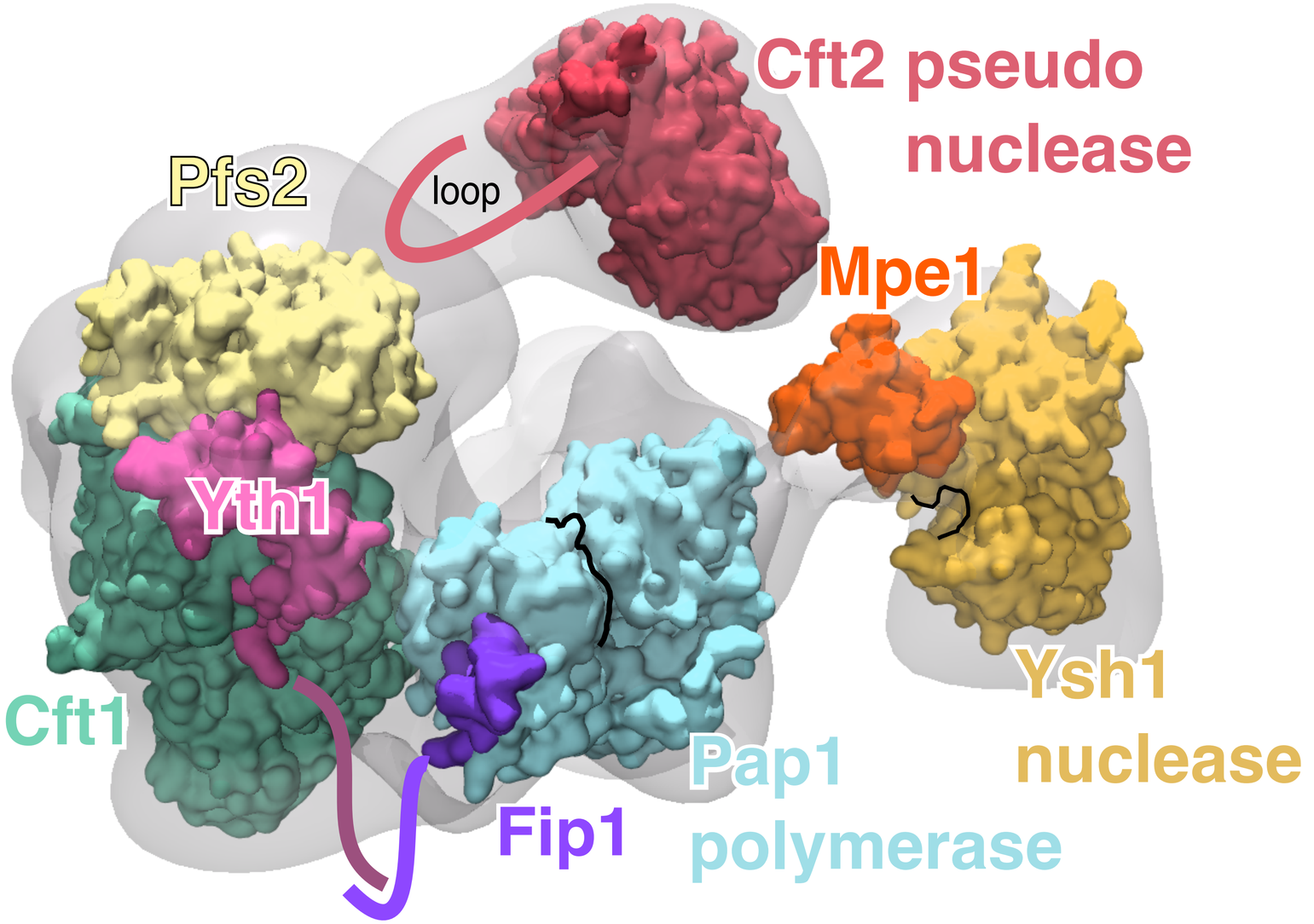




EMD-0324
Hill et al., Mol Cell 2019

Architecture of core CPF





Hill et al., Mol Cell 2019

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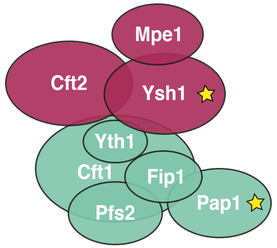
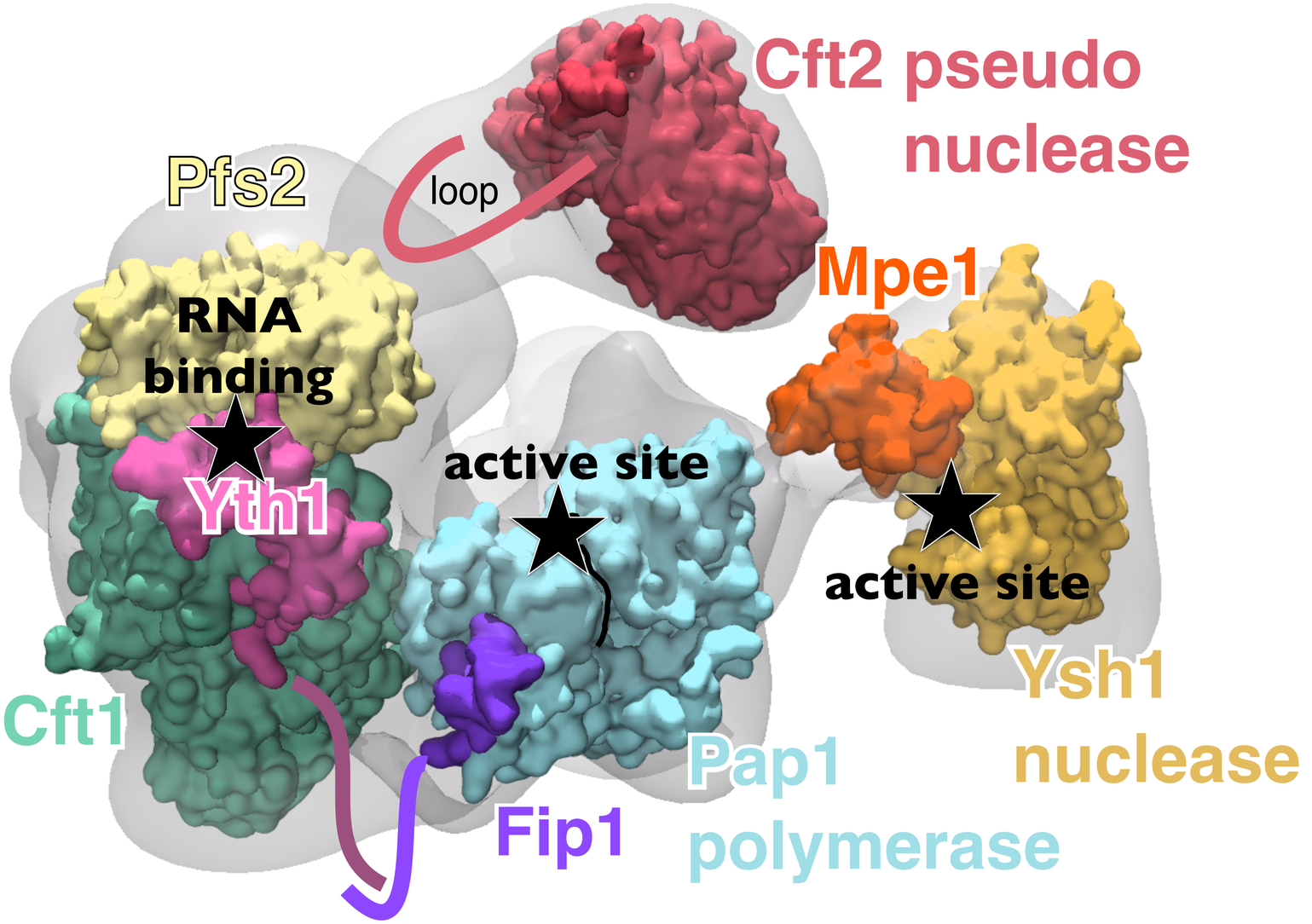
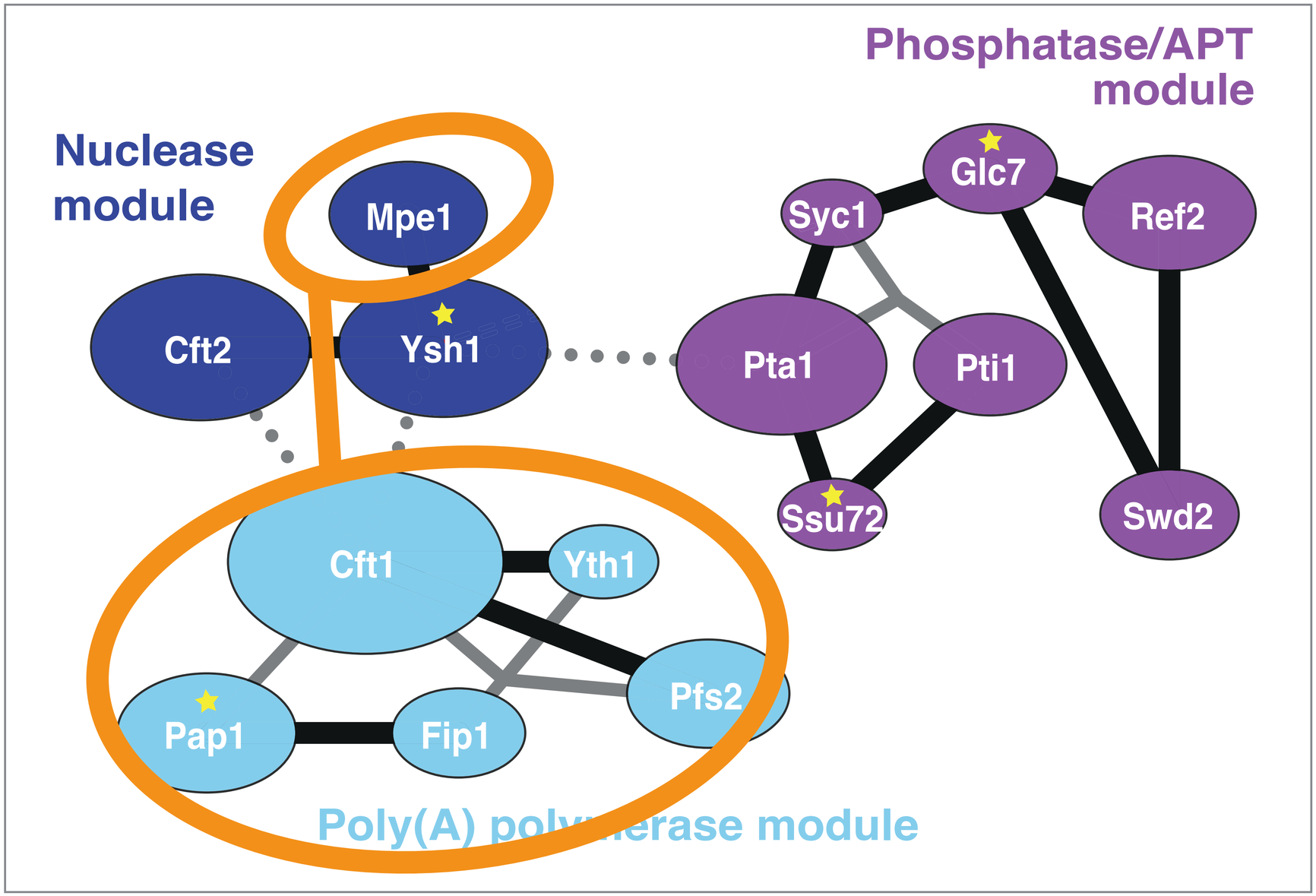




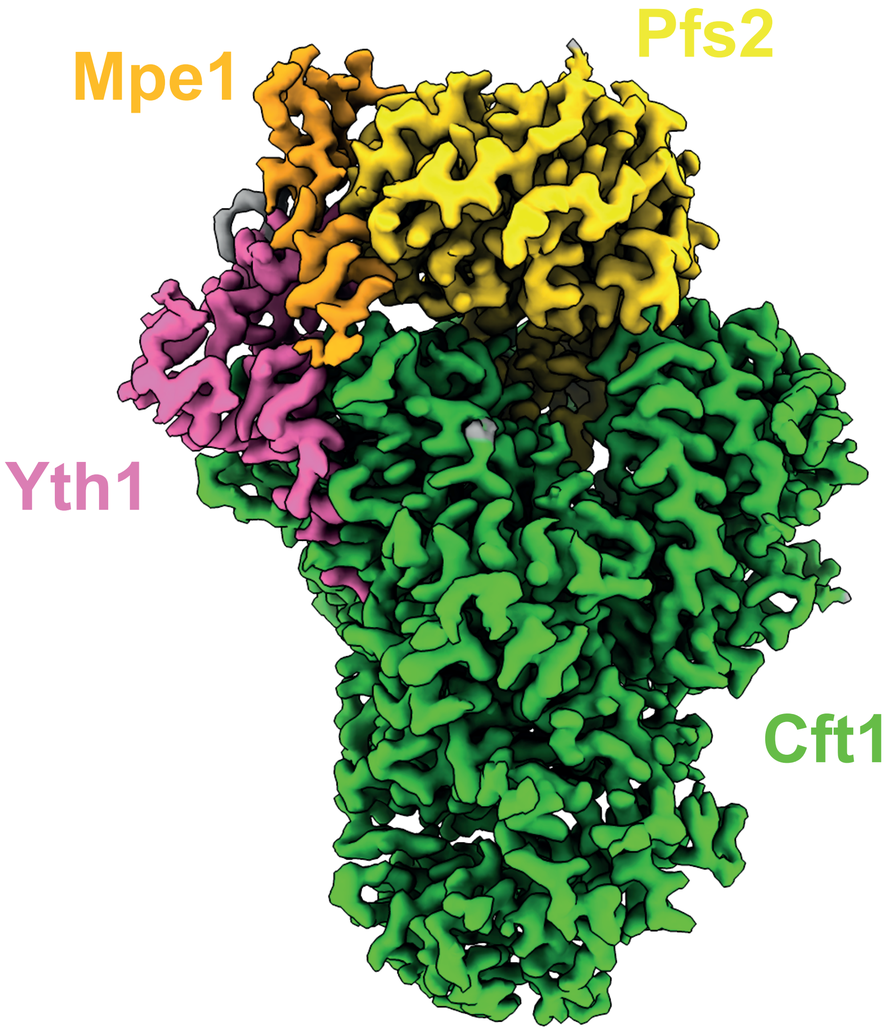
Hill et al., Mol Cell 2019

Juan Rodriguez-Molina



Vo et al 2001
Di Giammartino et al 2014
Lee and Moore 2014
Baejen et al 2014

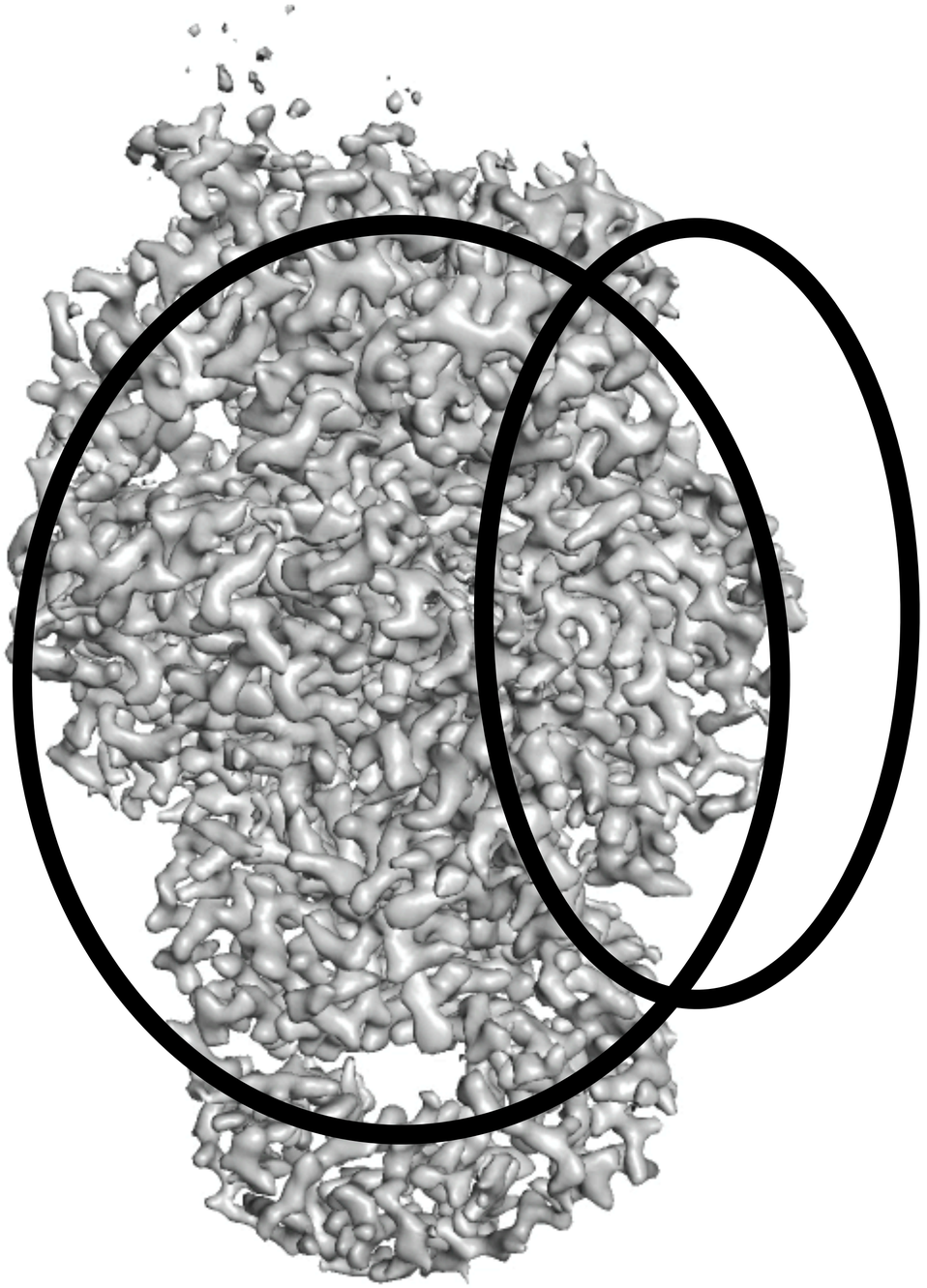
Mpe1 binds the polymerase module



2.7 Å resolution

Rodriguez-Mollina et al
Mol Cell 2022

RNA



Mpe1

Yth1

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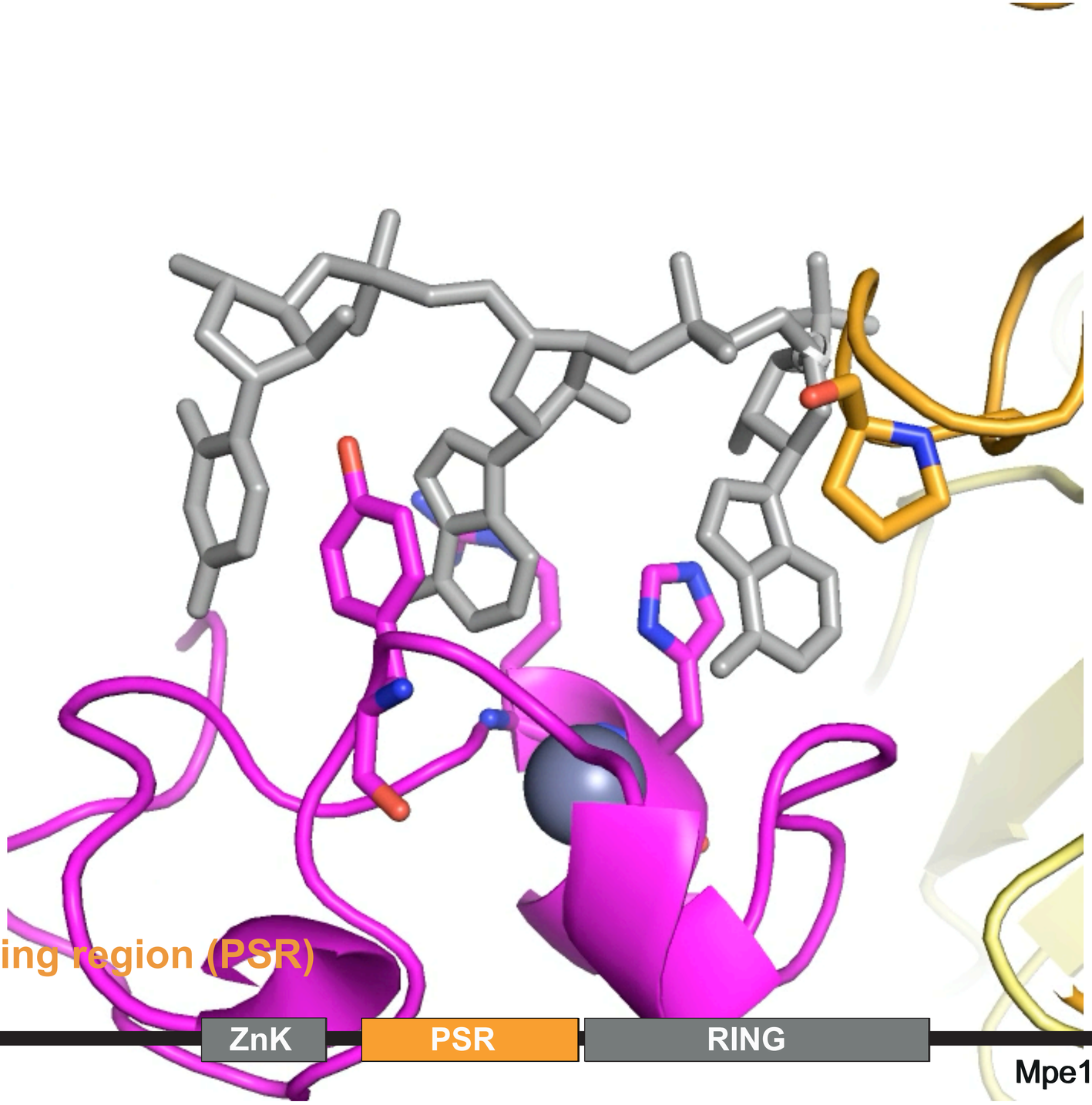

pre-mRNA sensing region (PSR)

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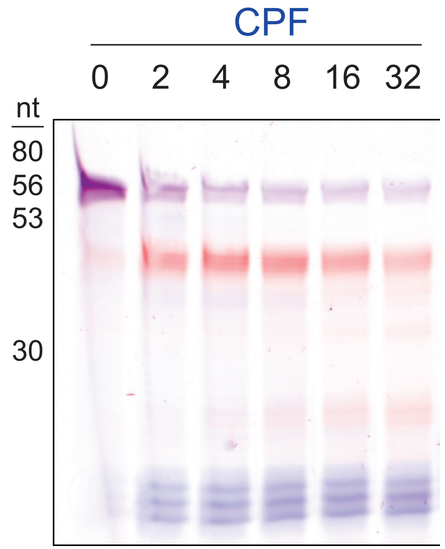
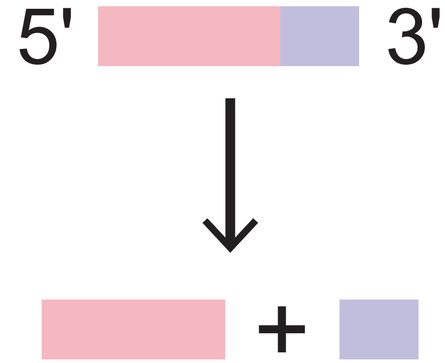
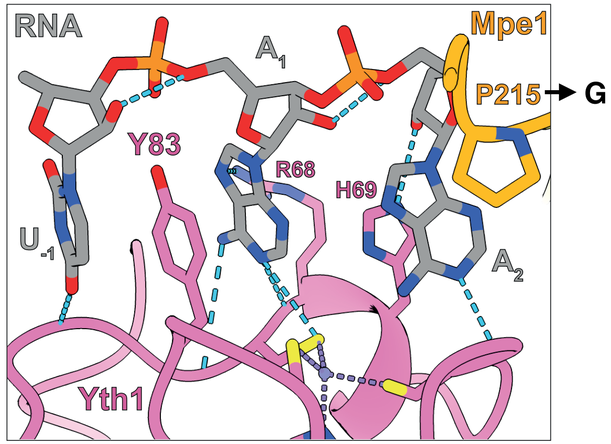
Mpe1 is important for cleavage







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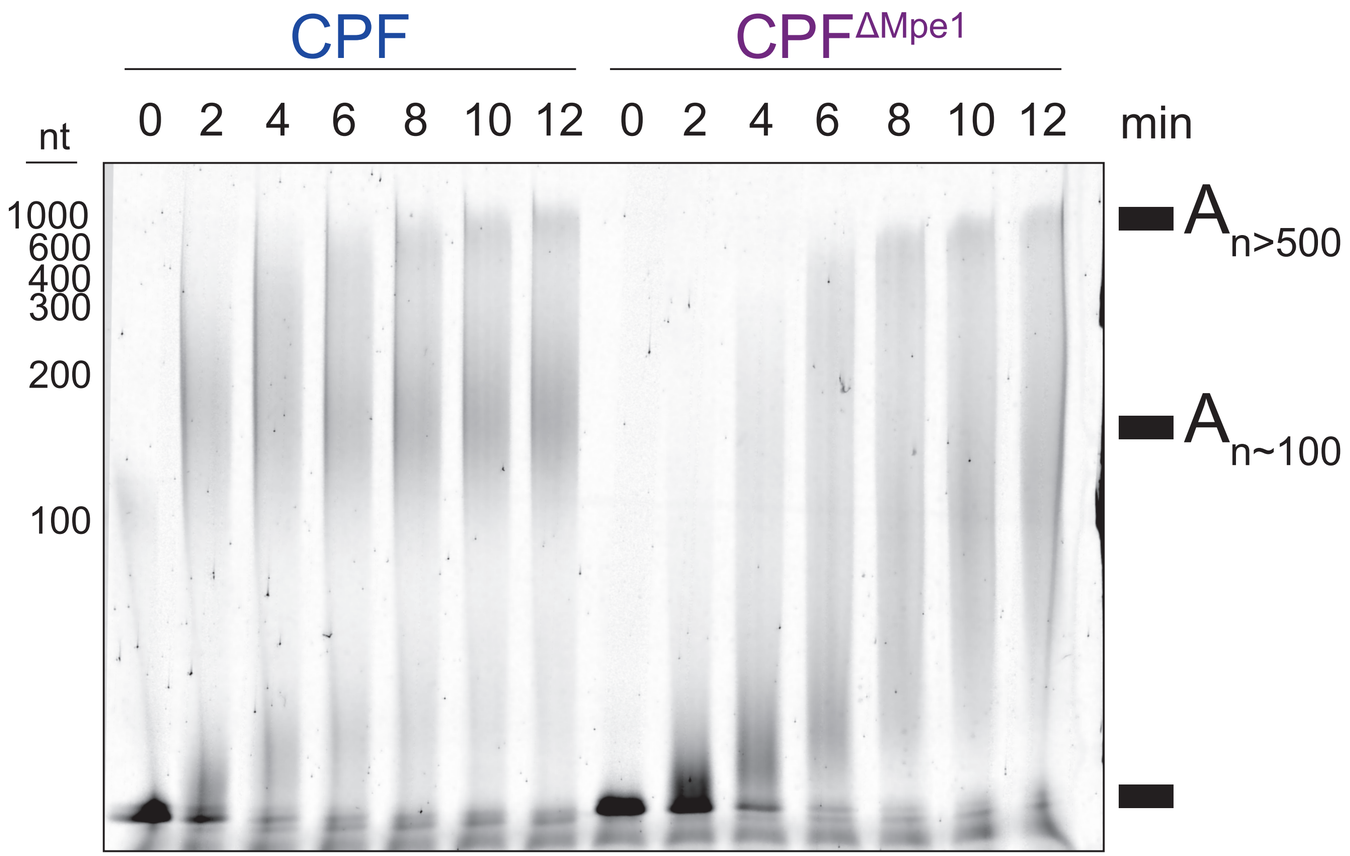
Mpe1 is important for cleavage and polyadenylation



Rodriguez-Mollina et al
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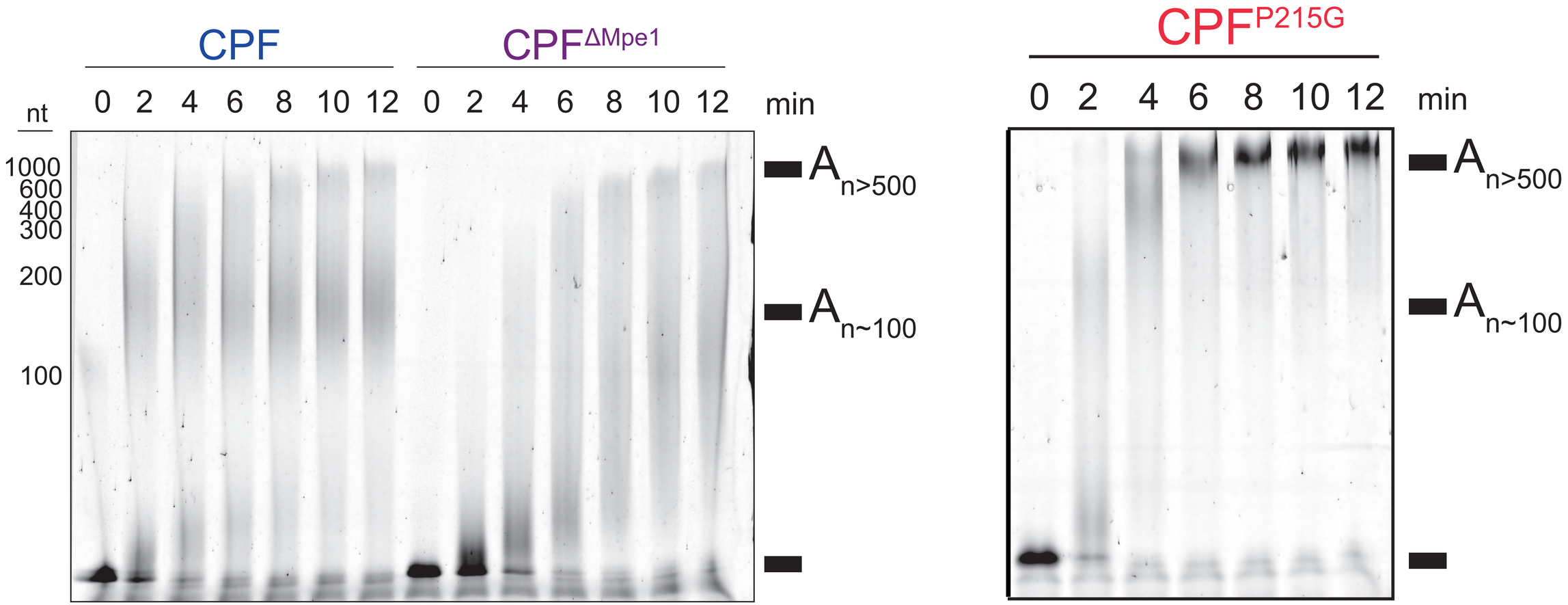
Mpe1 is important for cleavage and polyadenylation



Rodriguez-Mollina et al
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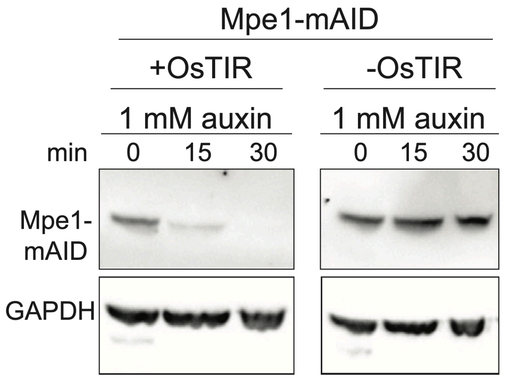
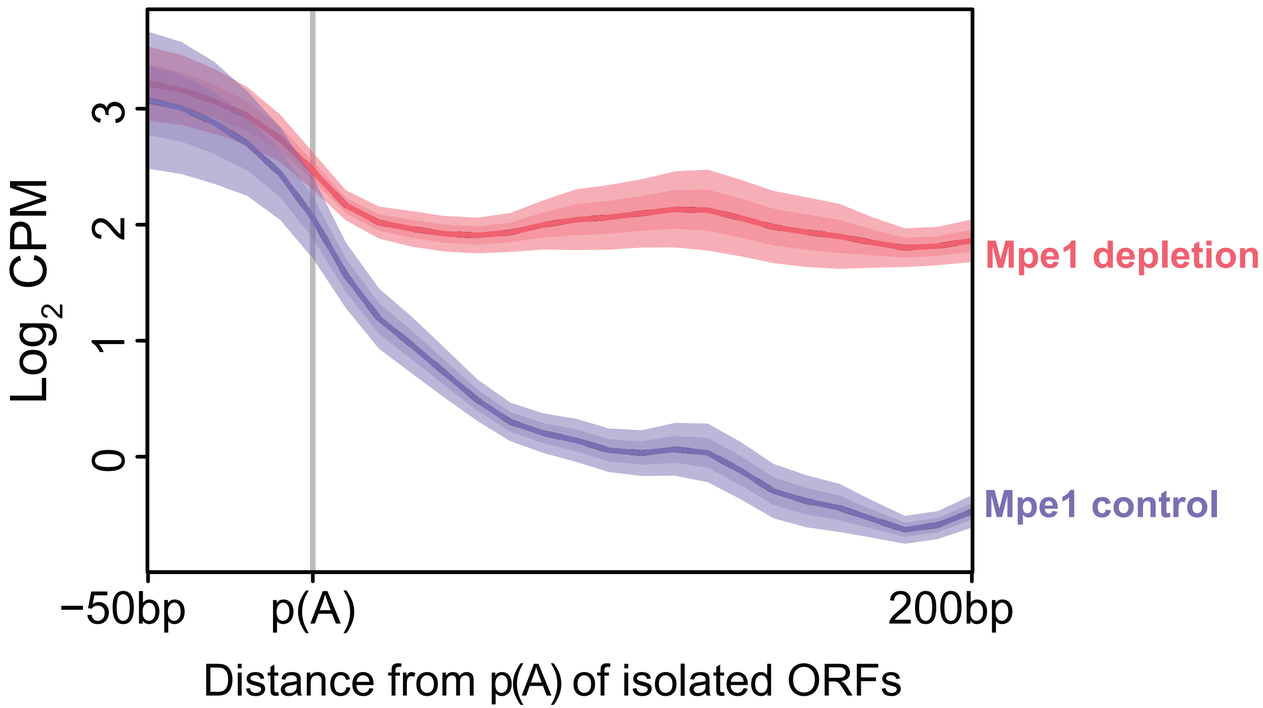
Mpe1 is required for transcription termination





Rodriguez-Mollina et al
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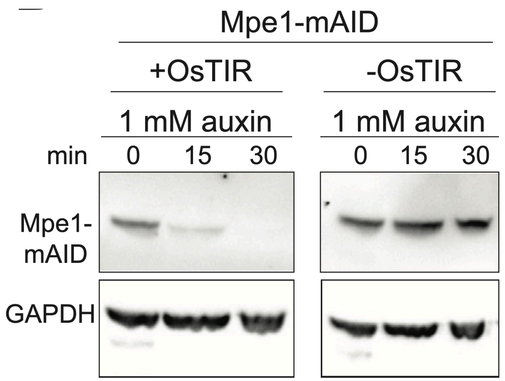

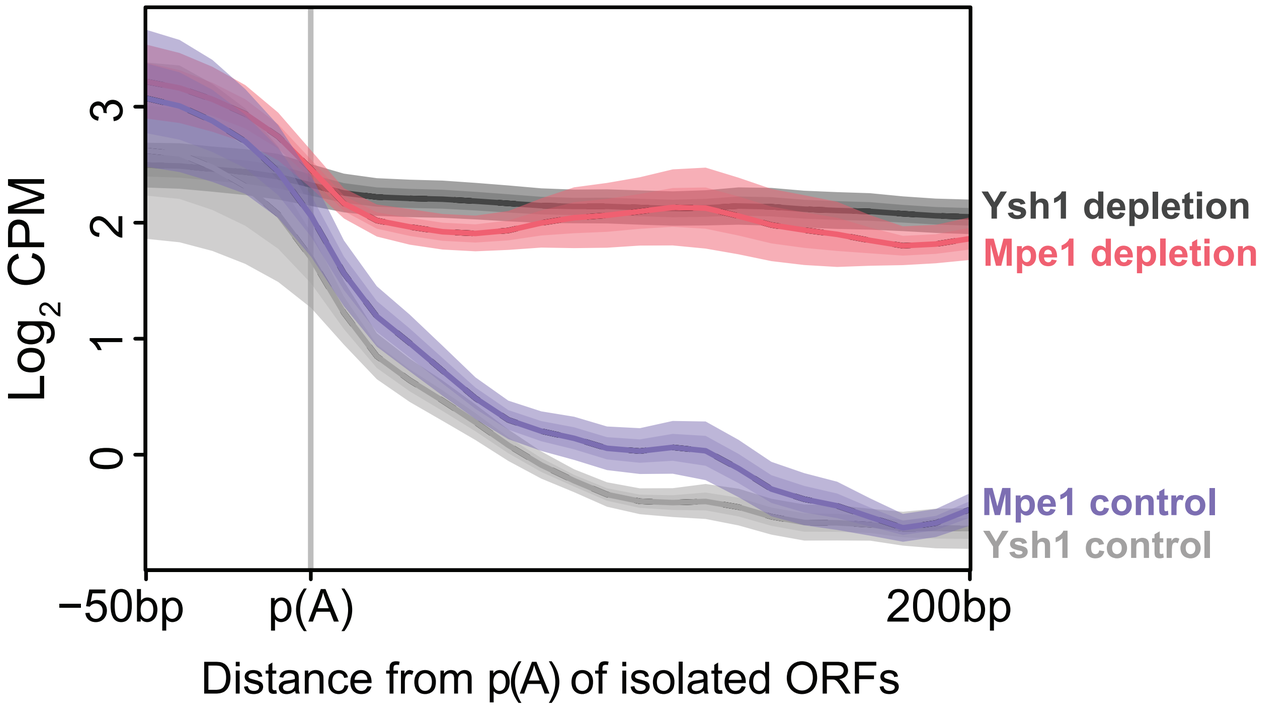

Baejen et al 2017

Rodriguez-Mollina et al
Mol Cell 2022

Dynamics likely regulate CPF activation

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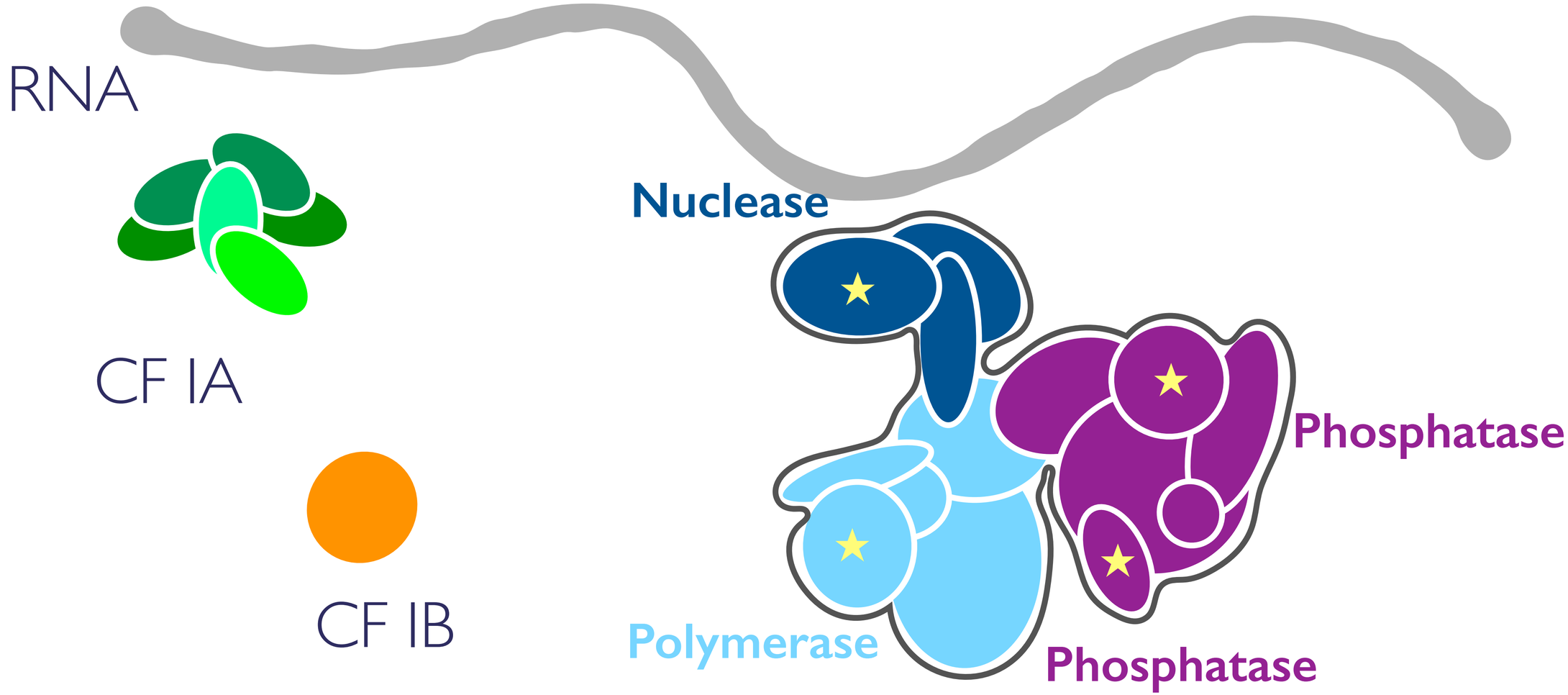
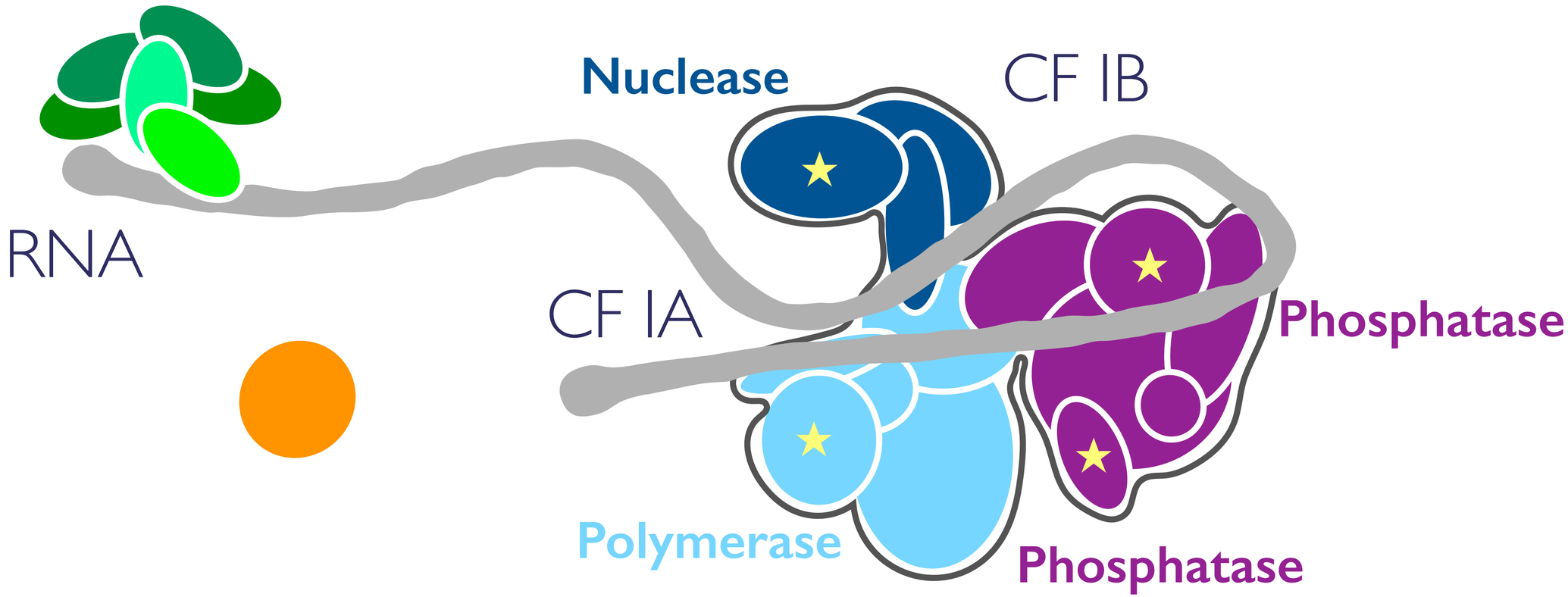


Dynamics likely regulate CPF activation



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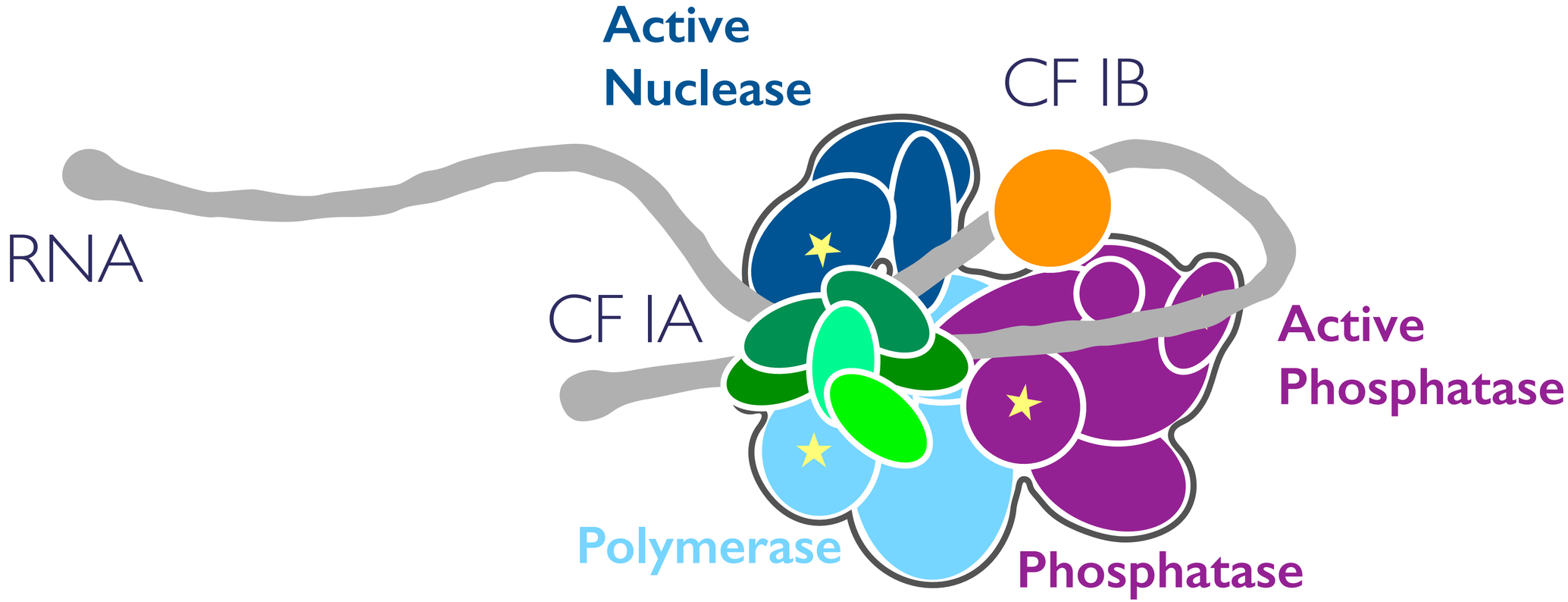
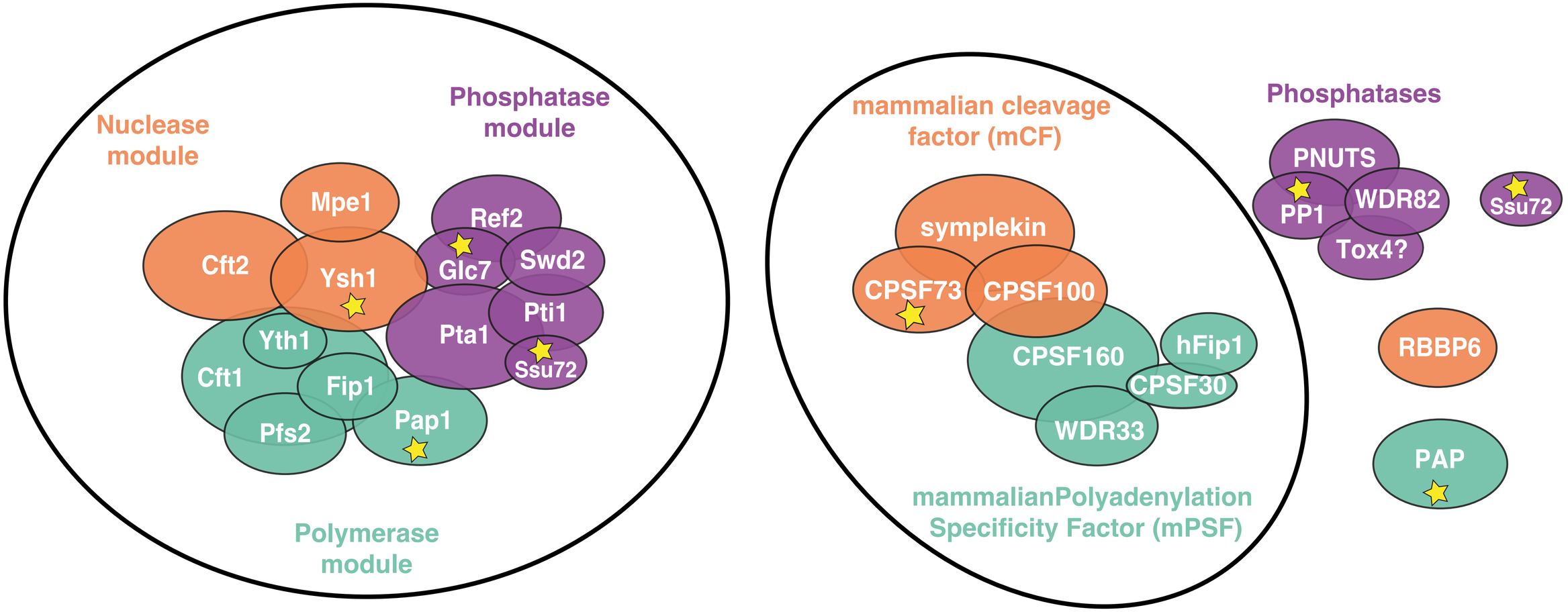


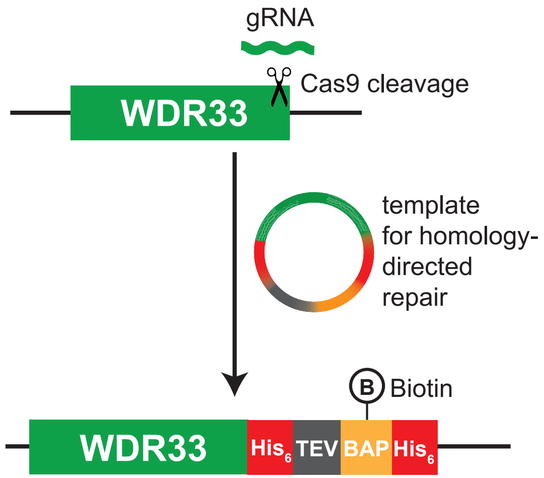
What about the human complex?



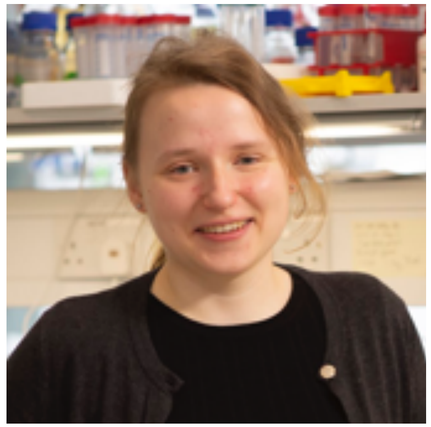
Yeast CPF

Human CPSF

RBBP6/Mpe1 is not a core subunit of human CPSF



Vytaute Boreikaite



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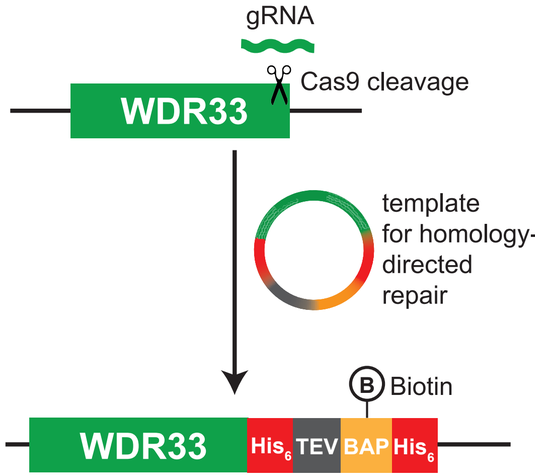
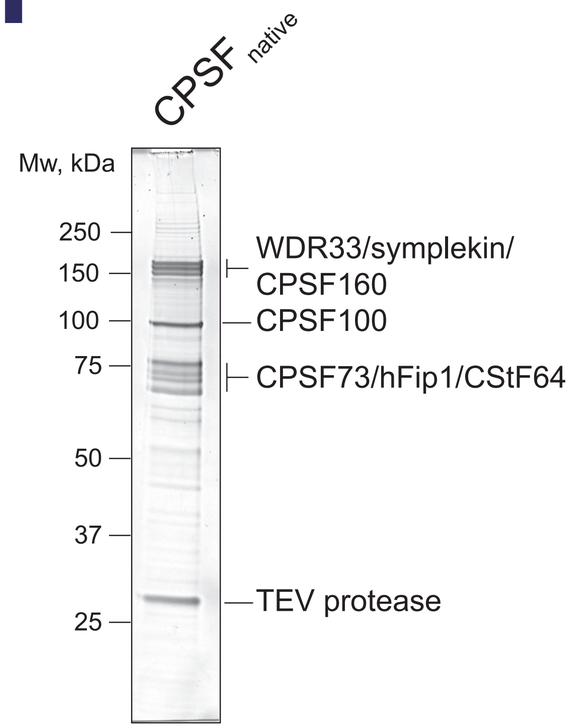
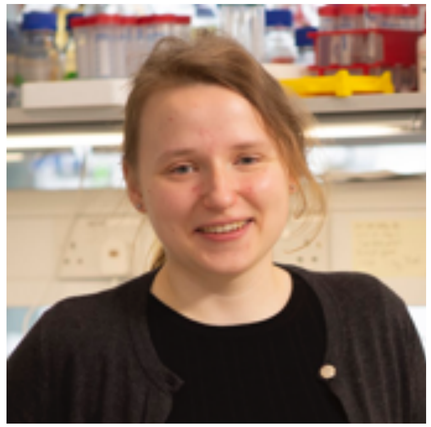
RBBP6/Mpe1 is not a core subunit of human CPSF





Vytaute Boreikaite



Boreikaite et al Genes Dev 2022

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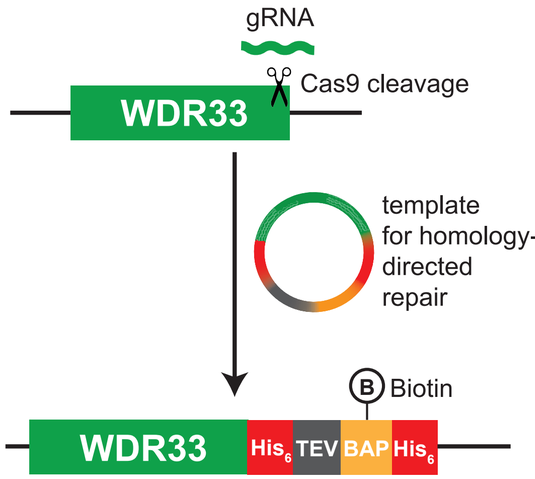
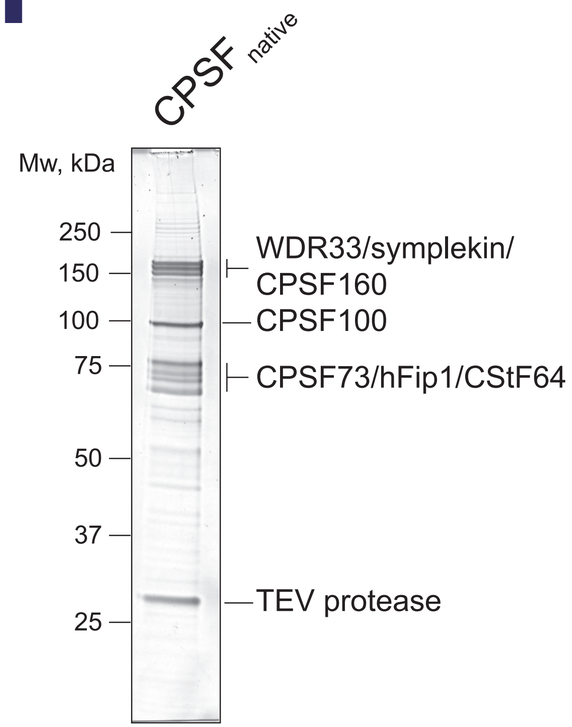
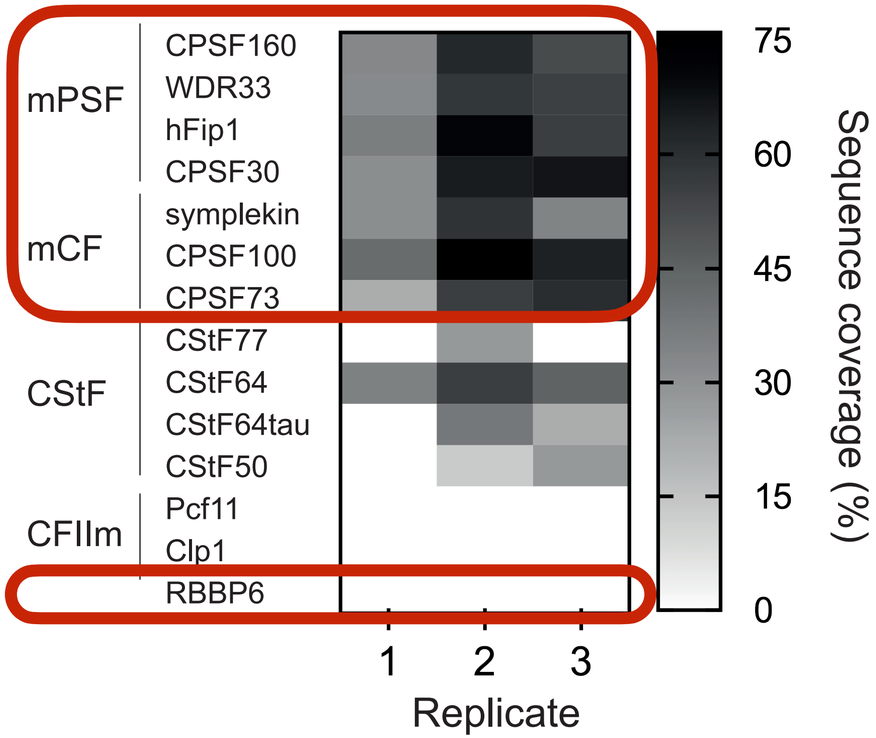
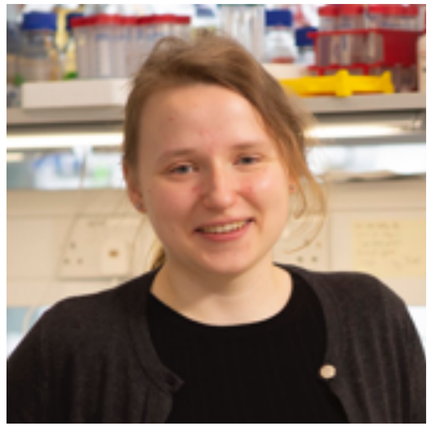






Vytaute Boreikaite



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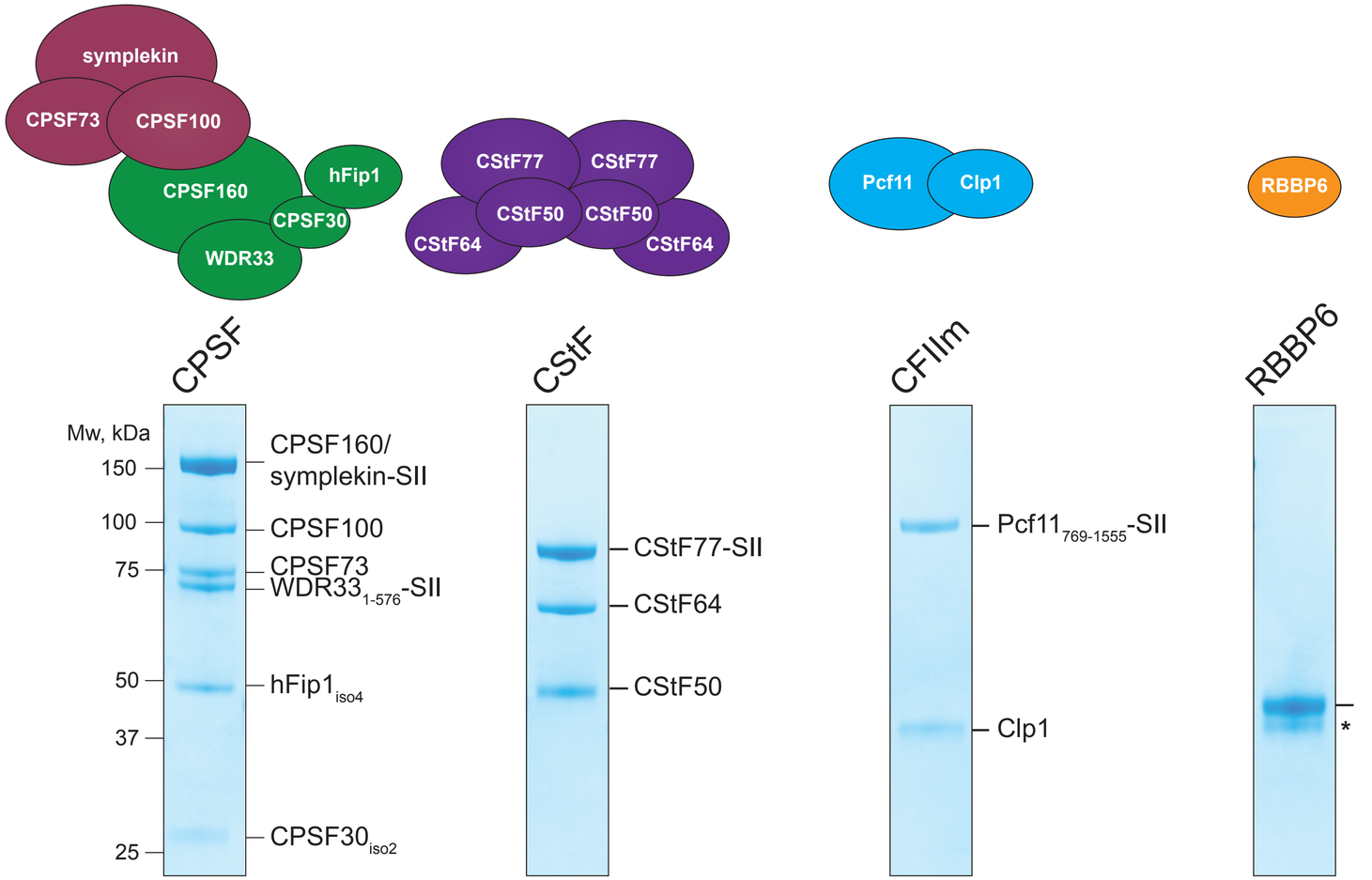
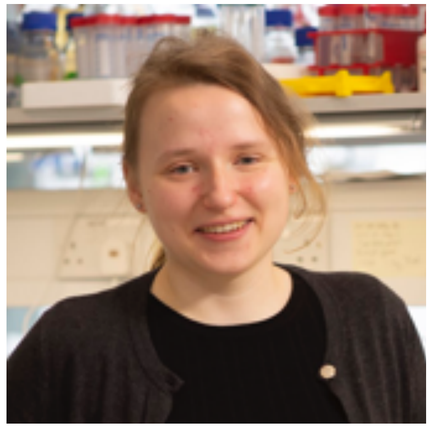
RBBP6 activates human pre-mRNA cleavage



Vytaute Boreikaite



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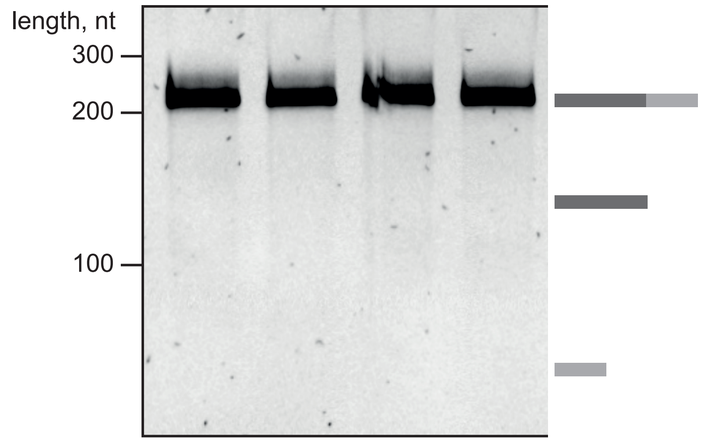
RBBP6 activates human pre-mRNA cleavage

CPSF	+	+	+	+
CStF	-	+	-	+
CFIIm	-	-	+	+
RBBP6	-	-	-	-



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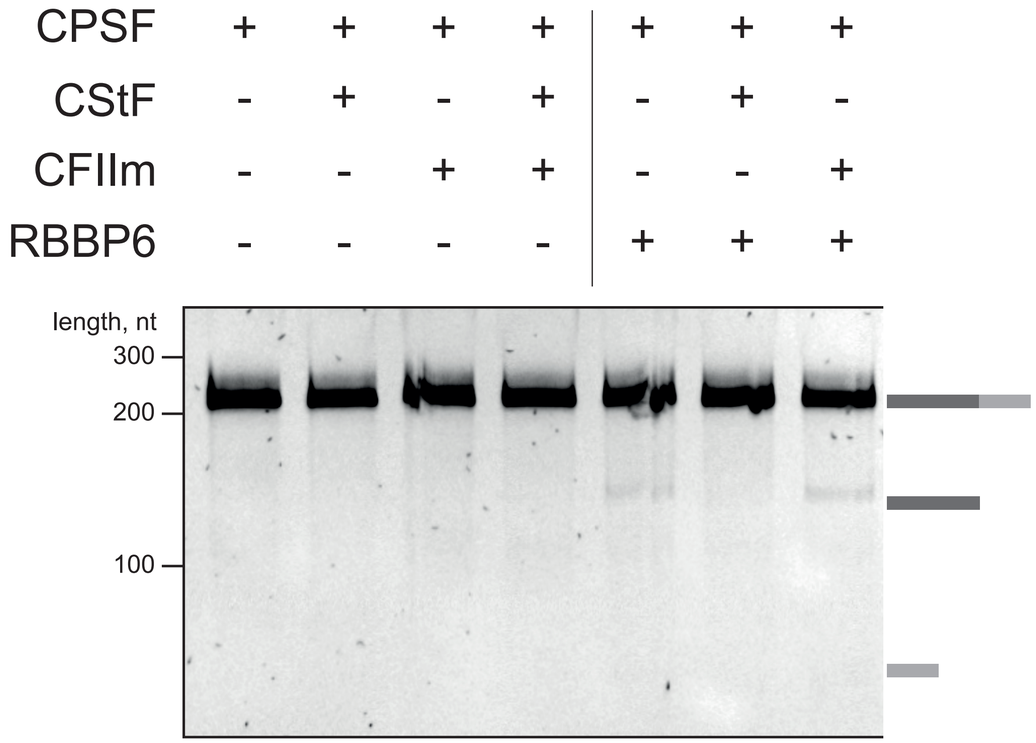
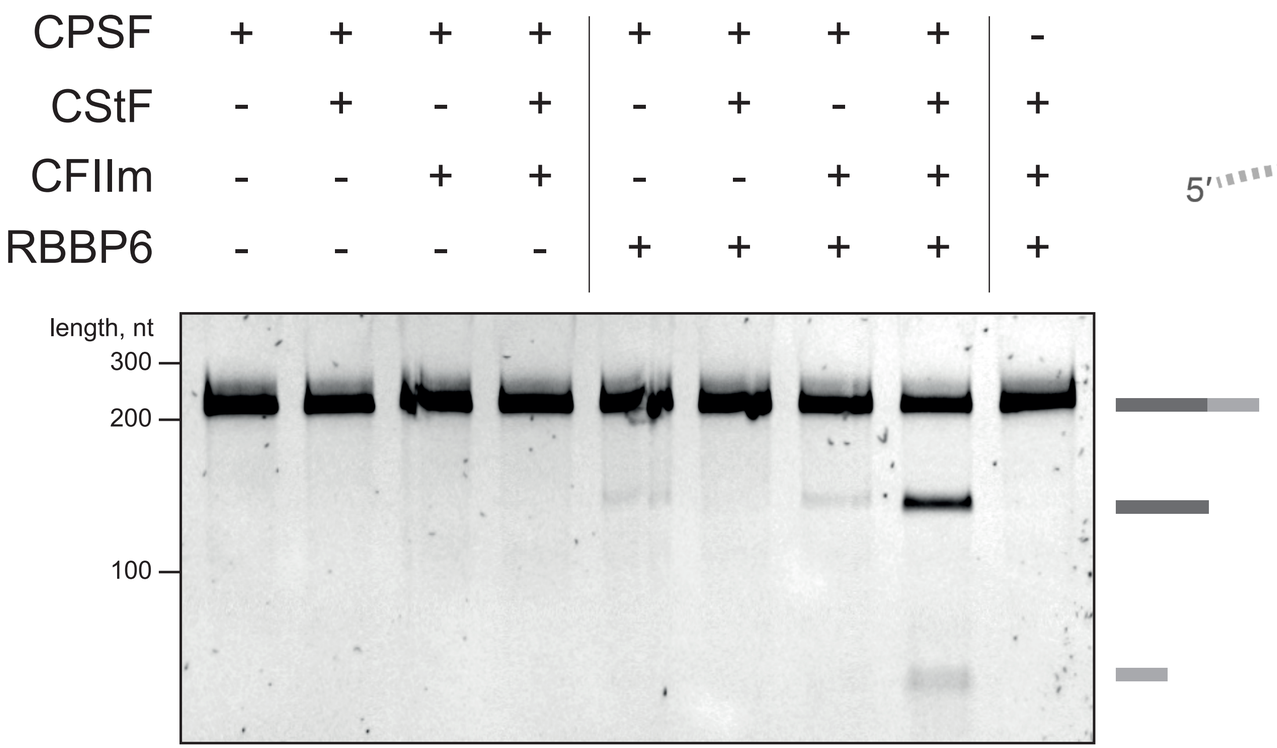
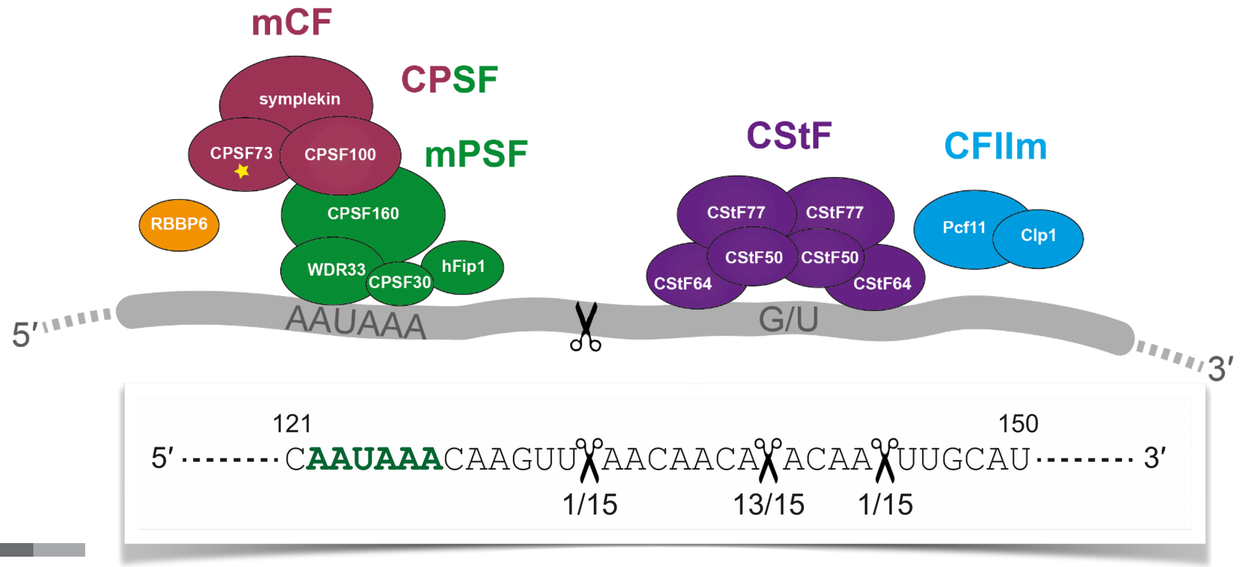


Boreikaite et al Genes Dev 2022

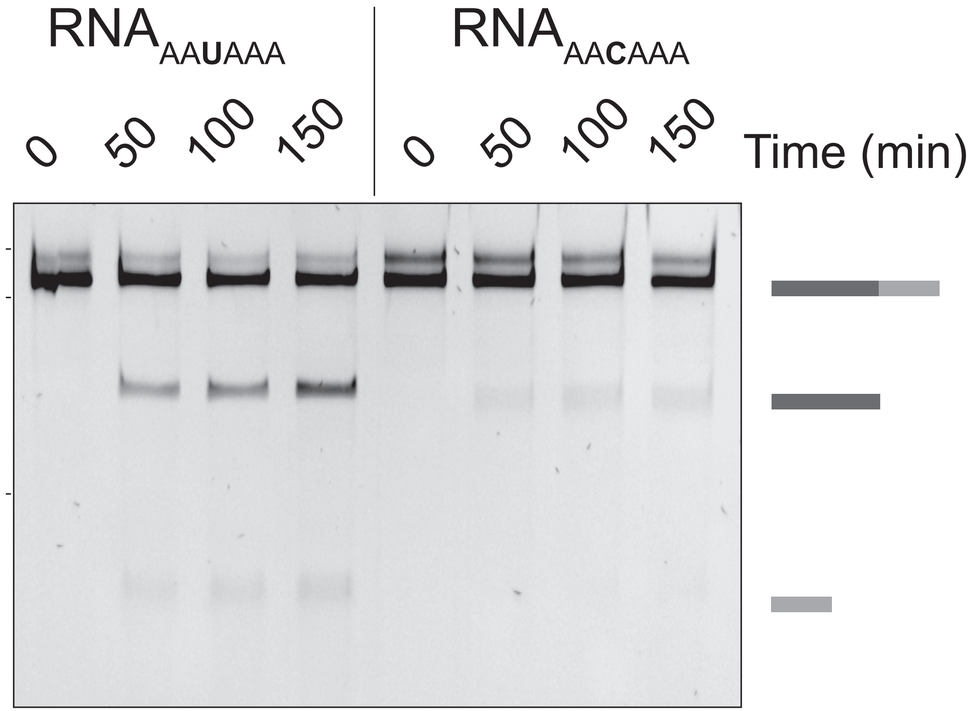
RBBP6 activates human pre-mRNA cleavage

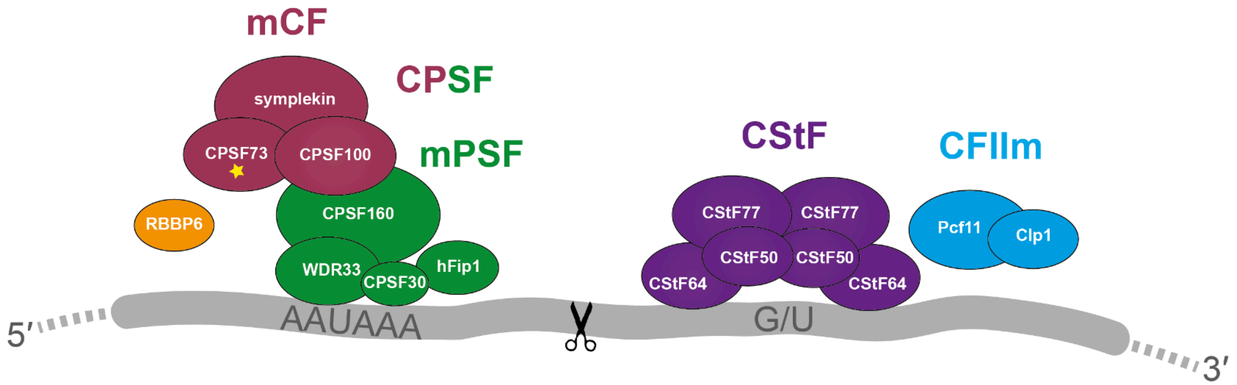




Boreikaite et al Genes Dev 2022

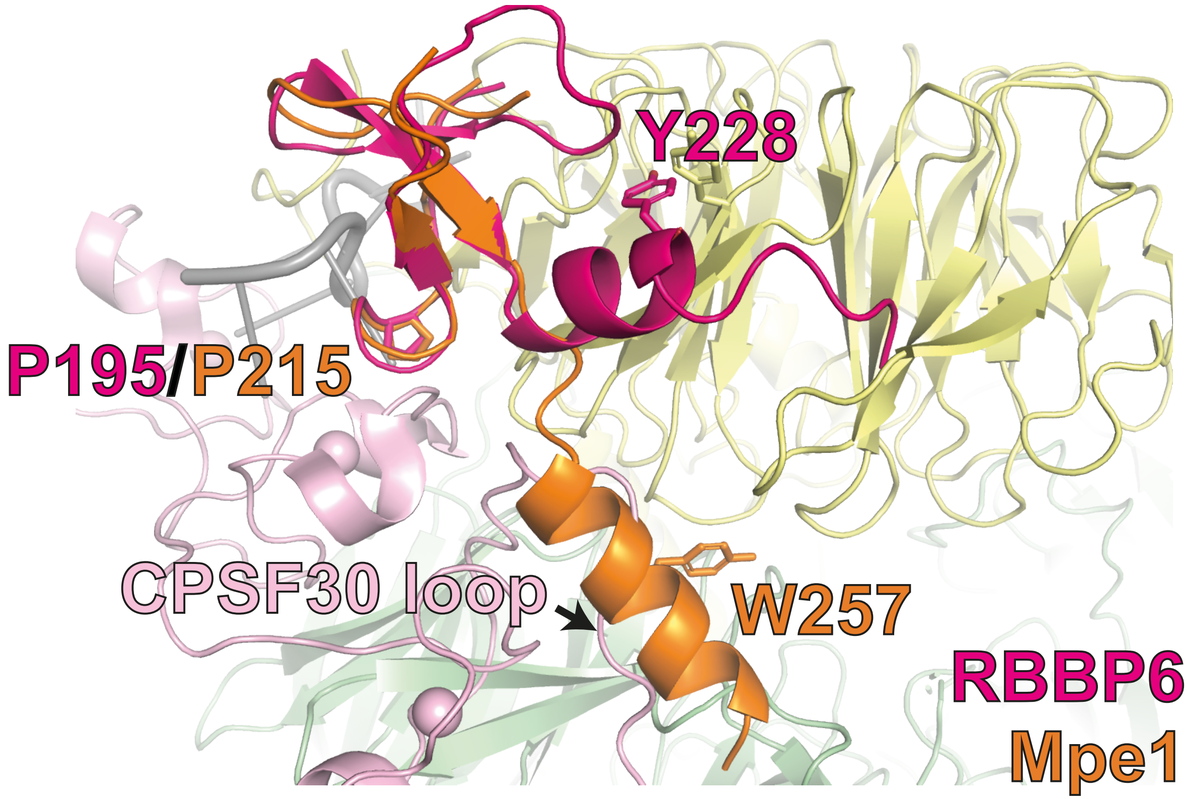
RBBP6 activates human pre-mRNA cleavage





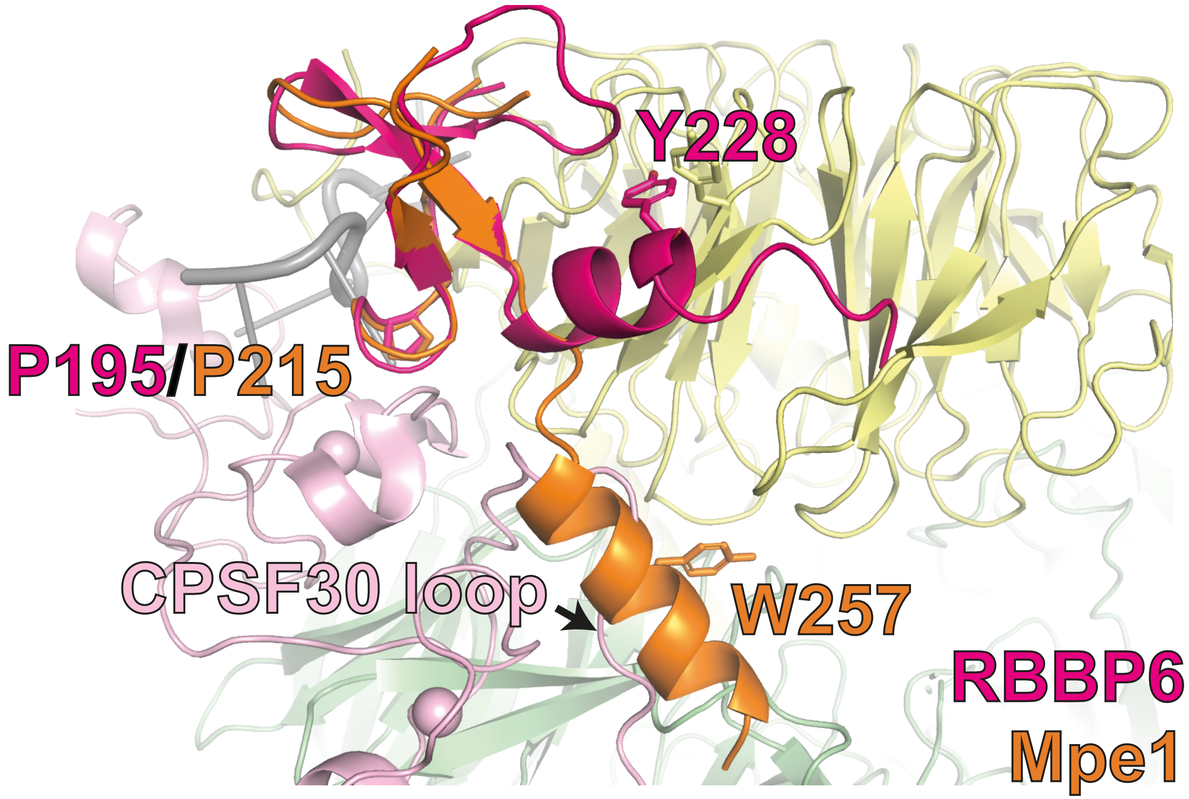
Boreikaite et al Genes Dev 2022

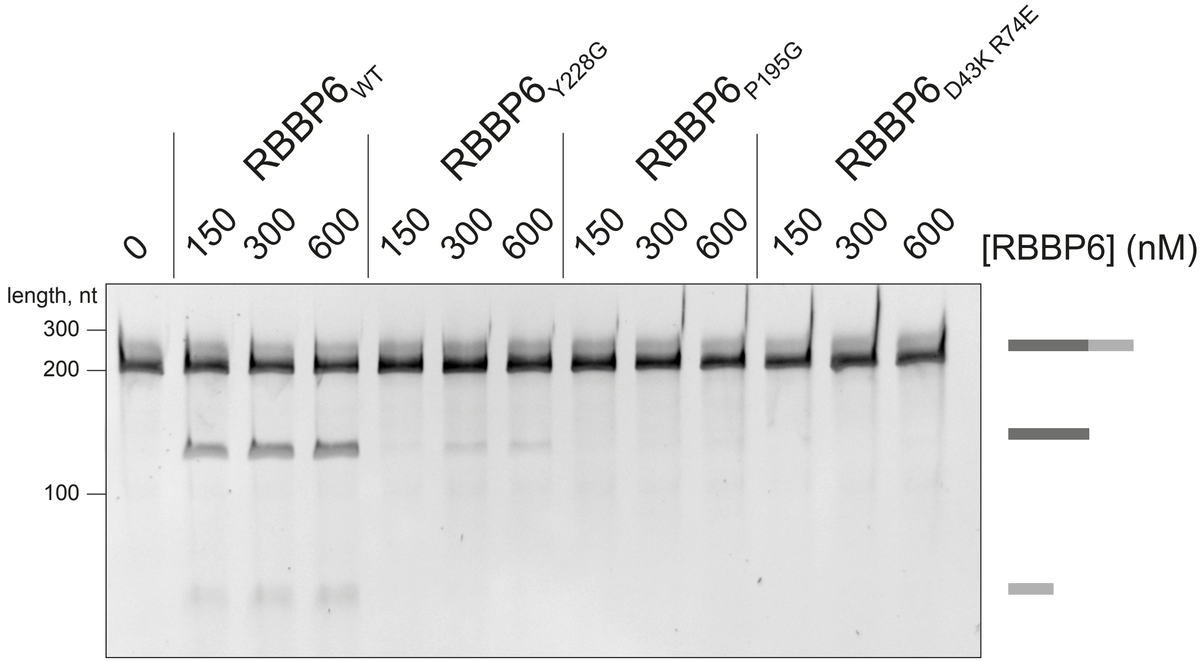
RBBP6 activates human pre-mRNA cleavage



Boreikaite et al Genes Dev 2022

RBBP6 activates human pre-mRNA cleavage





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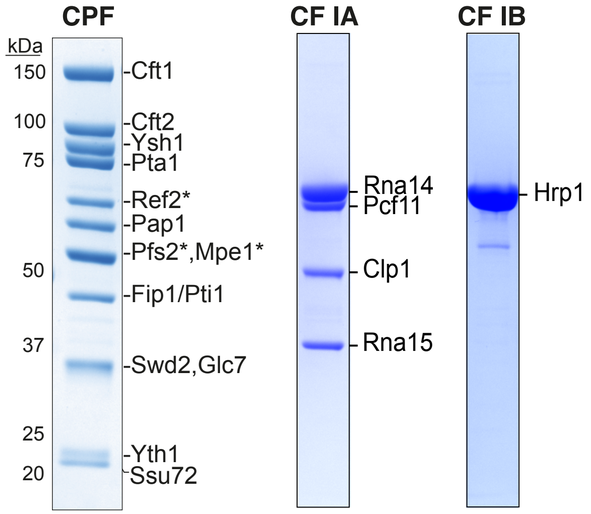
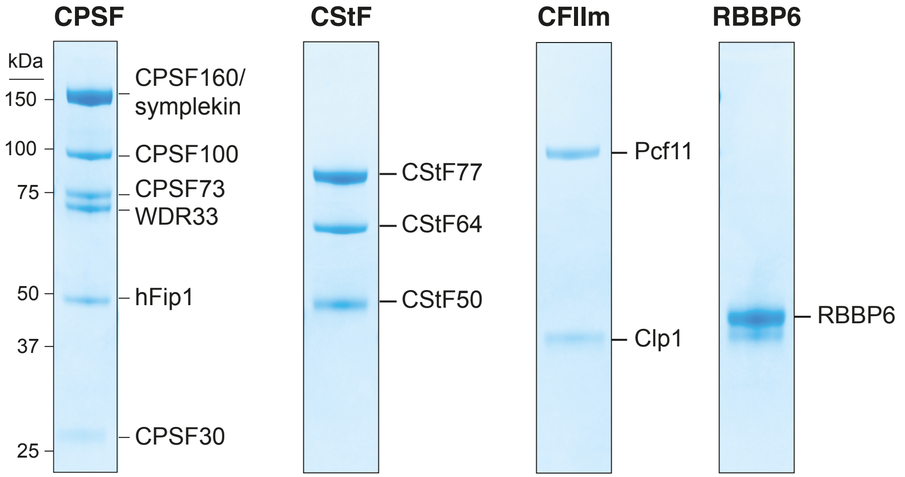
Reconstitution of 3'-end processing

Yeast





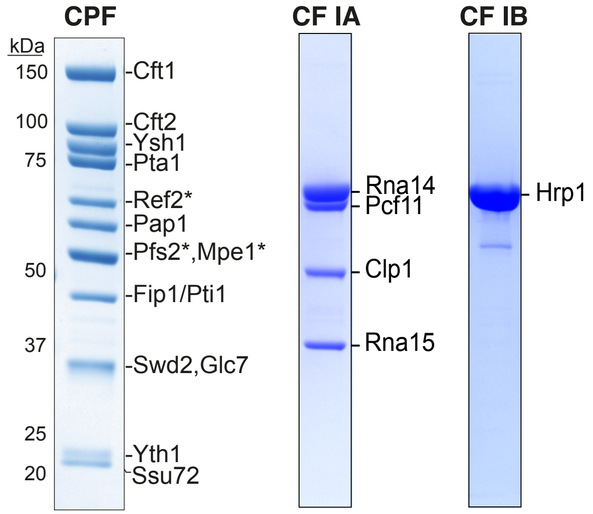
Human

Hill et al, *Mol Cell* 2019

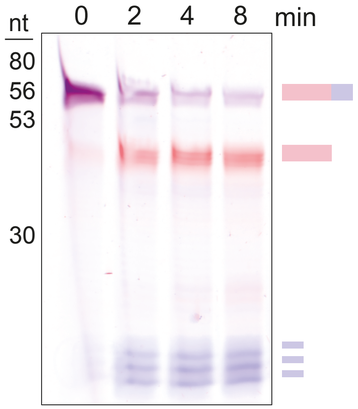
Boreikaite et al, G&D 2022

Reconstitution of 3'-end processing

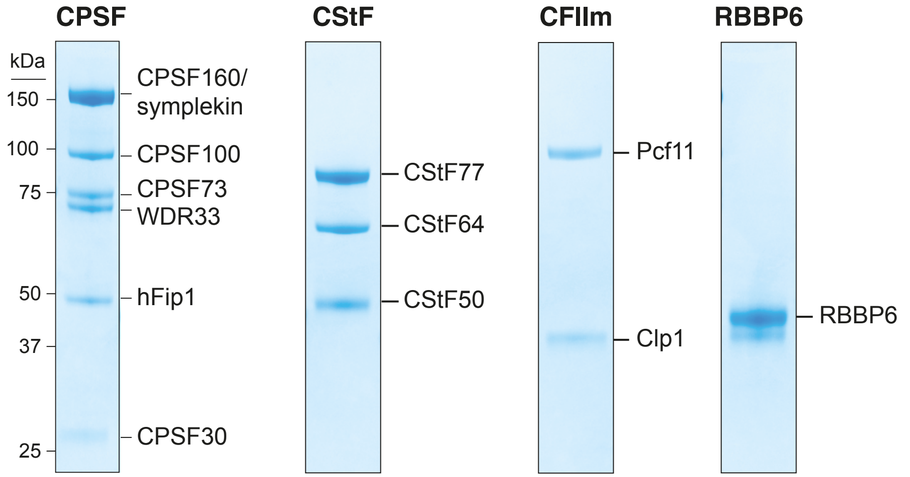
Yeast



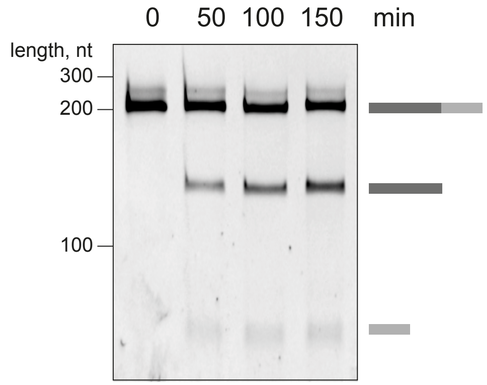
Cleavage assay



Human



Cleavage assay



Hill et al, *Mol Cell* 2019

Boreikaite et al, G&D 2022

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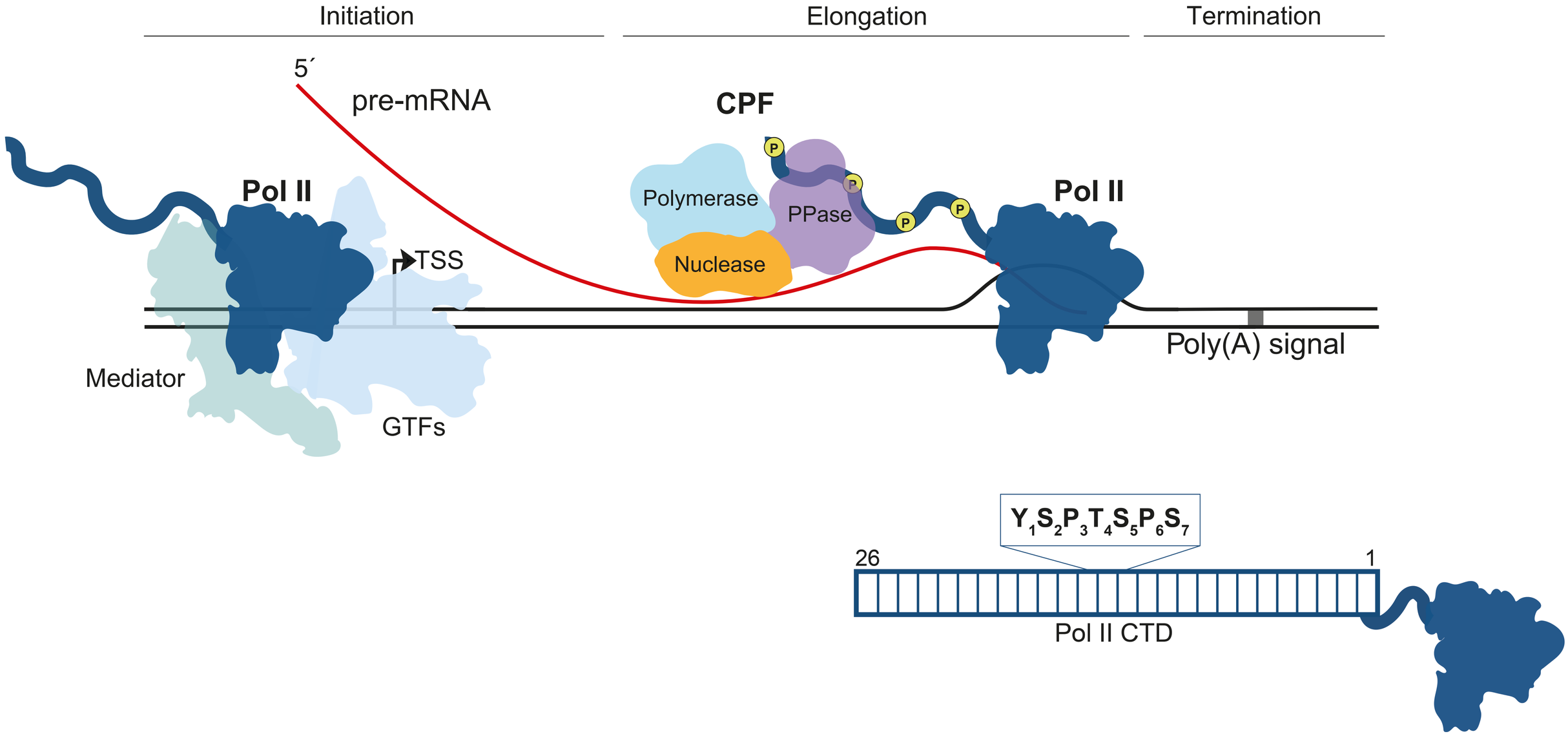
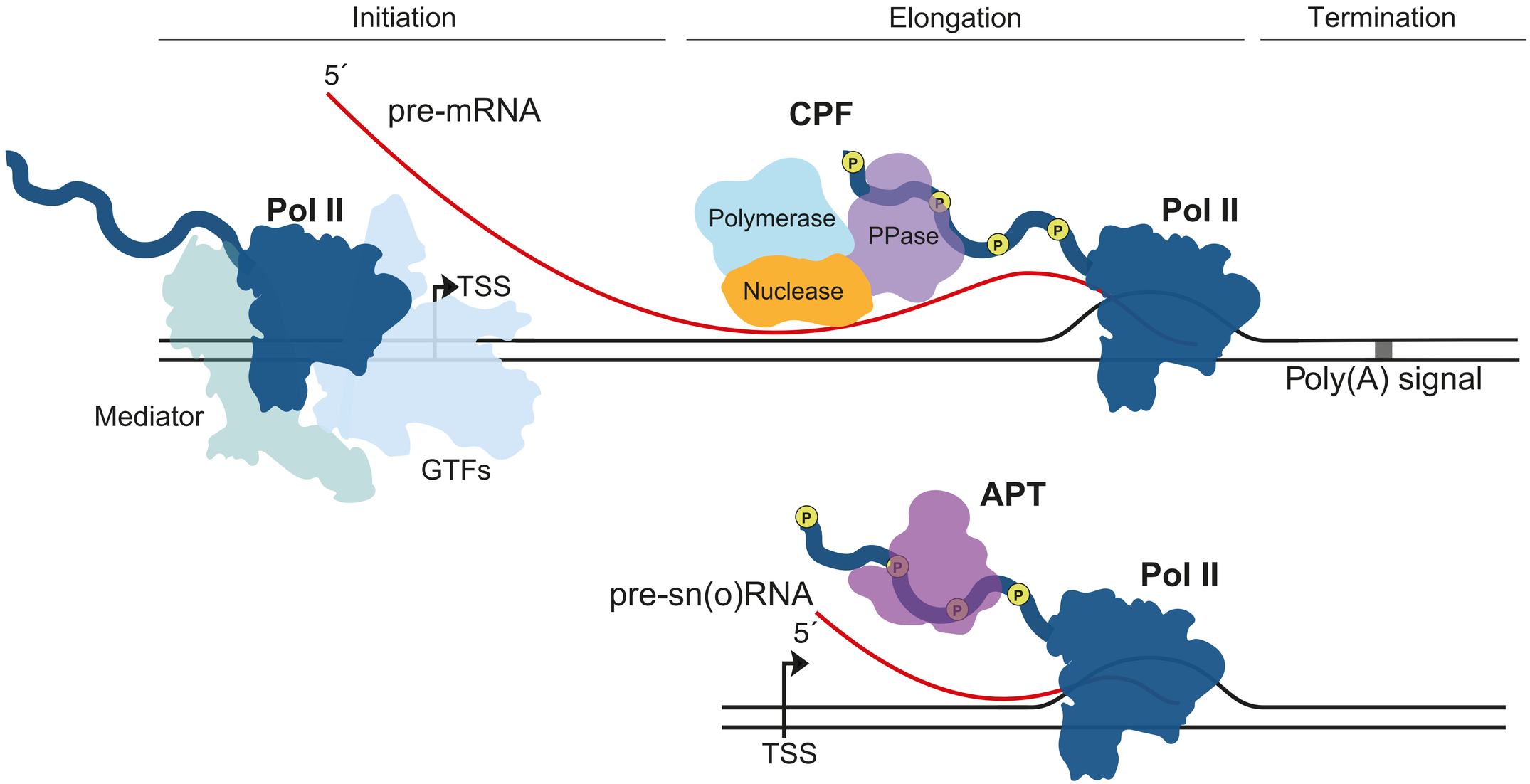
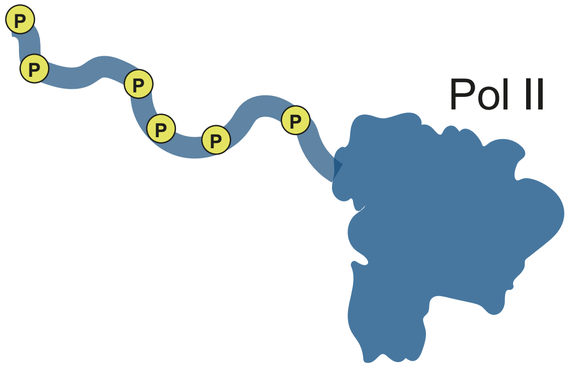
How does CPF interact with Pol II?

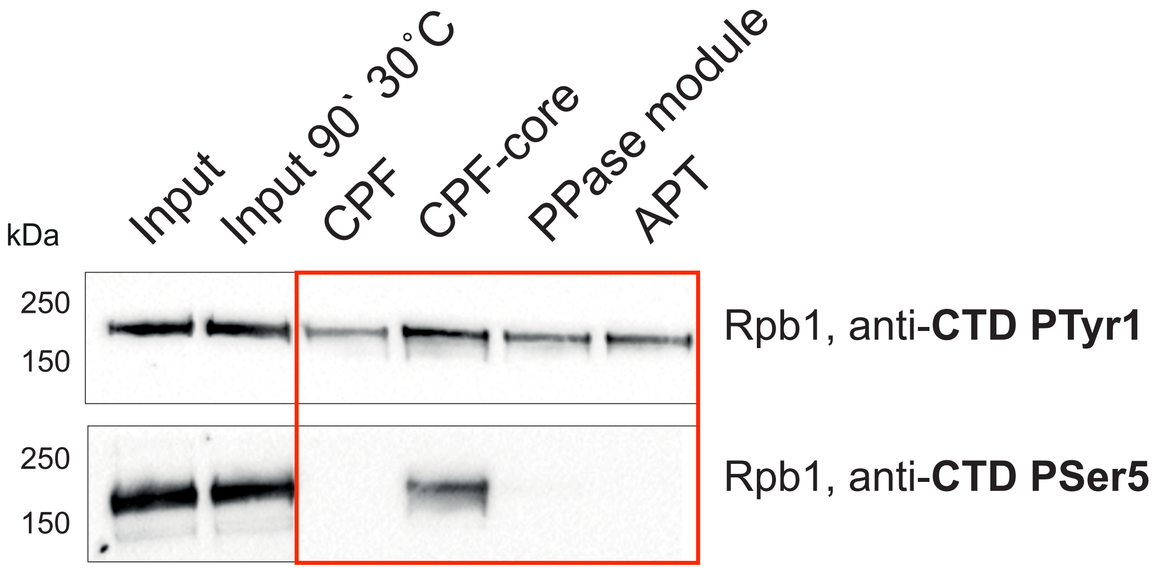


Pol II CTD phosphorylation

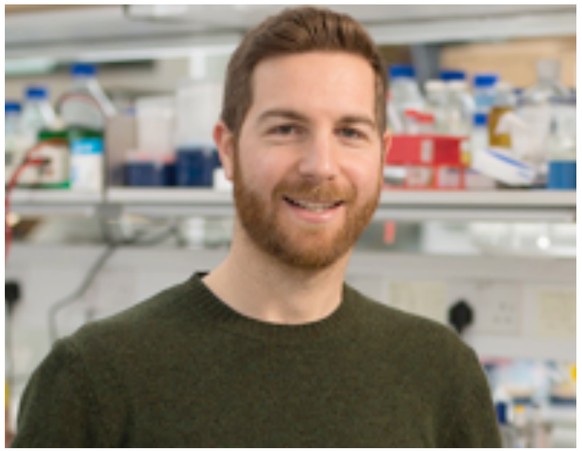


CPF and APT dephosphorylate Pol II CTD

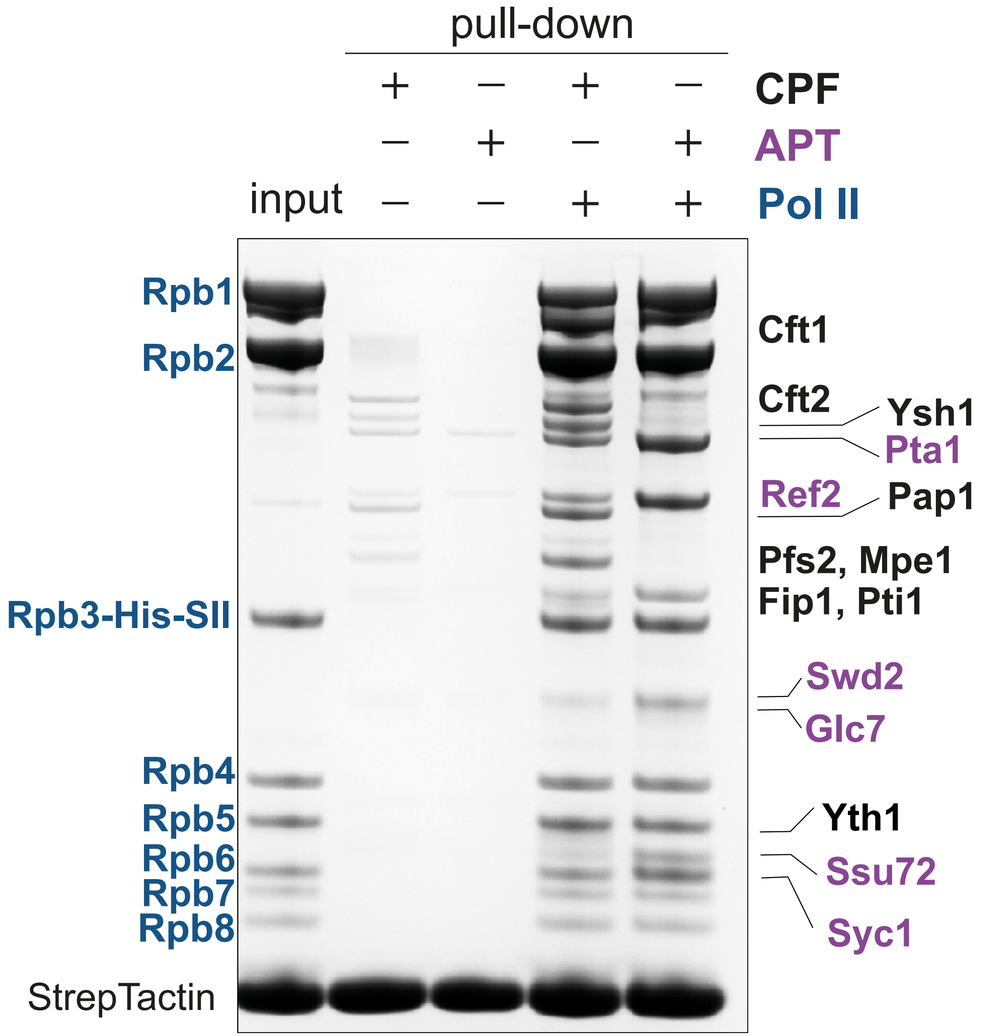




Manuel Carminati



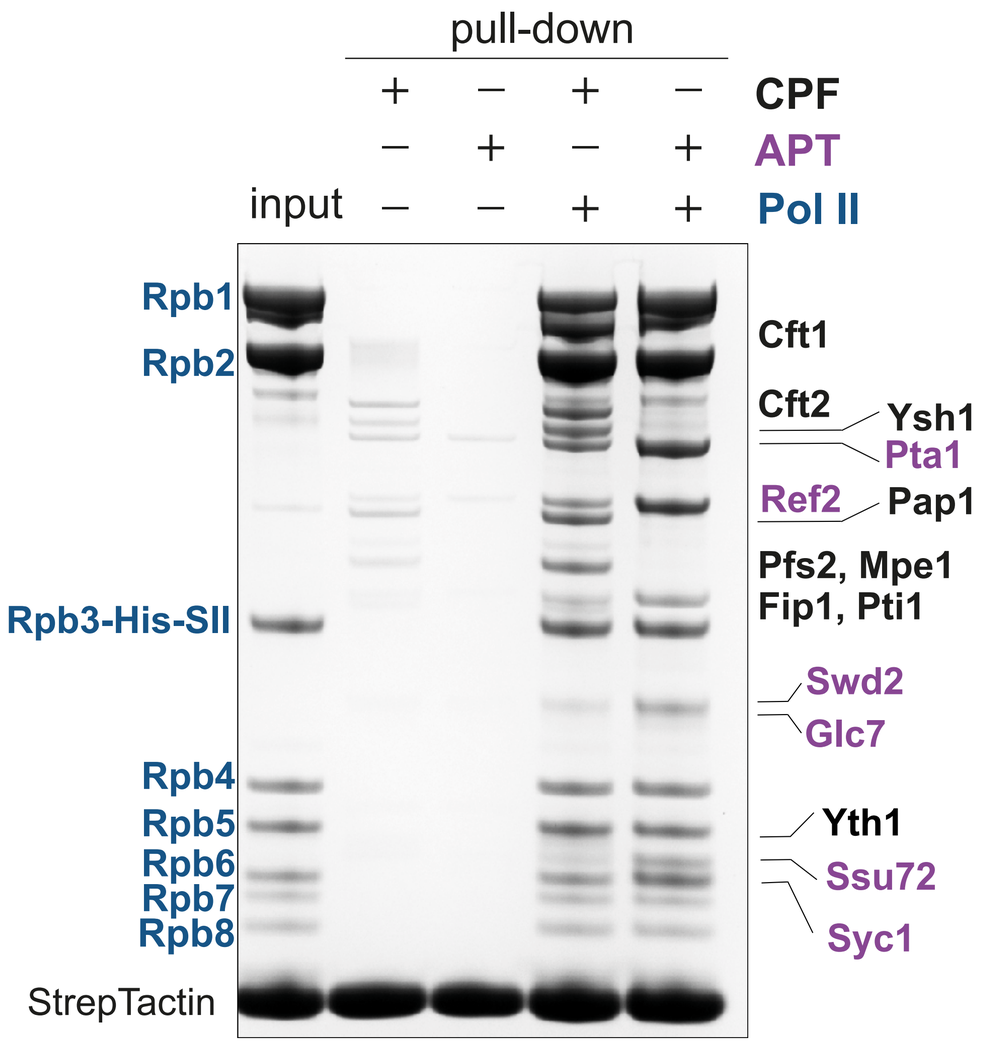
CPF and APT interact directly with Pol II

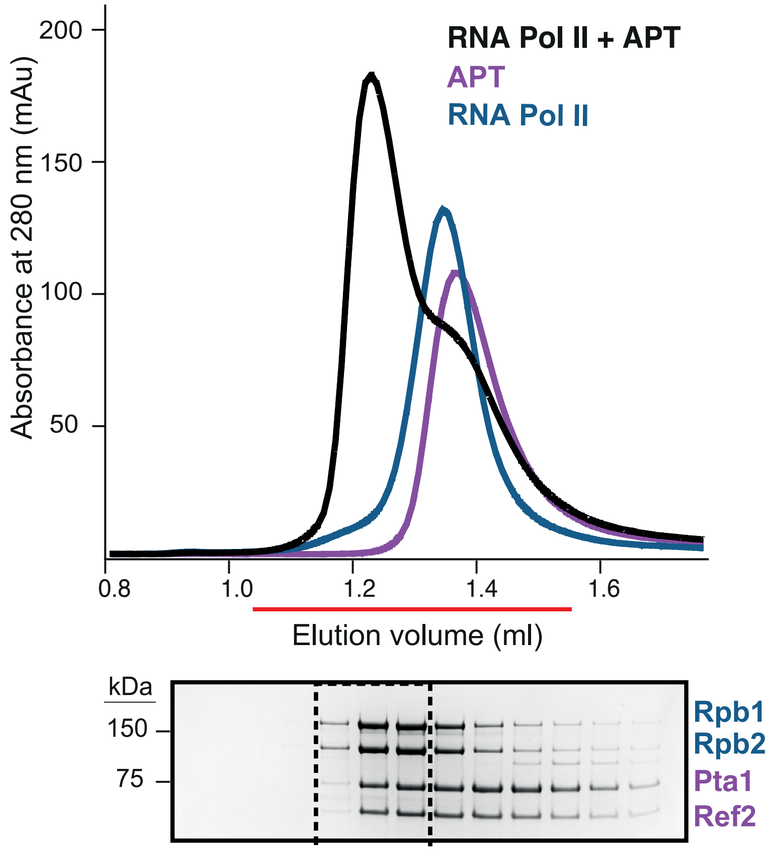


Phosphatase module is necessary and sufficient for the interaction

Carminati et al, bioRxiv

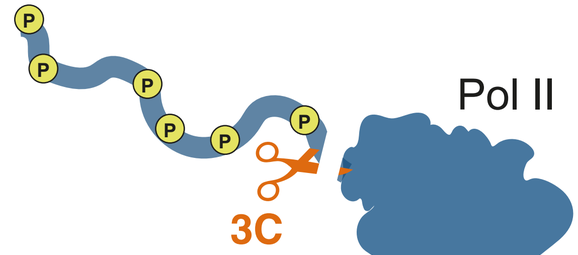
CPF and APT interact directly with Pol II

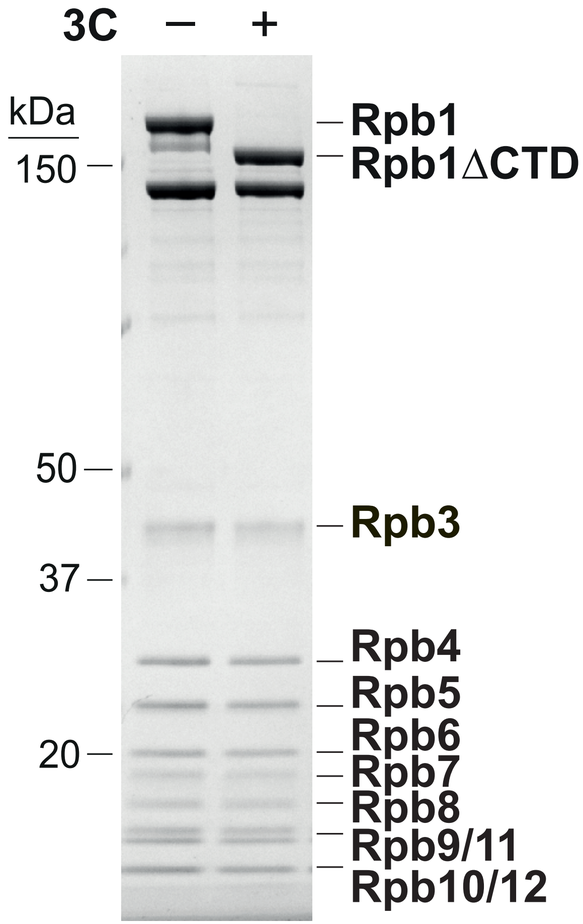


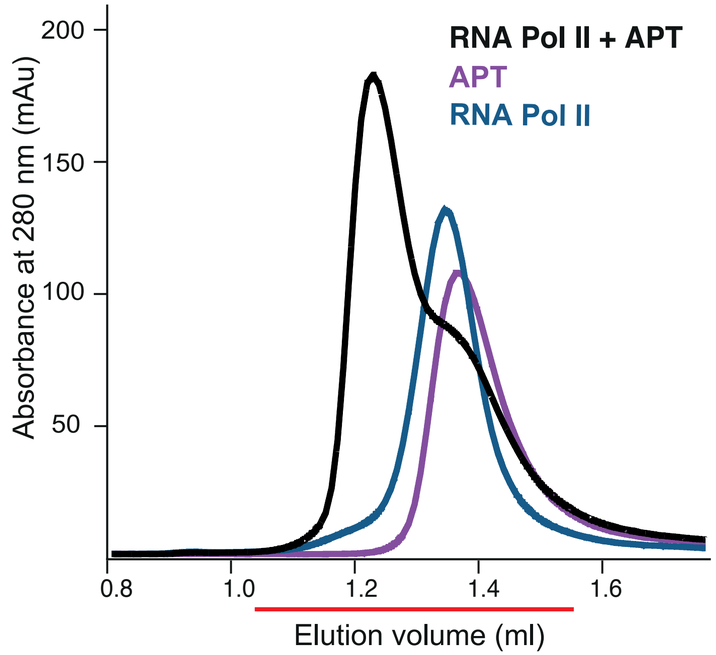


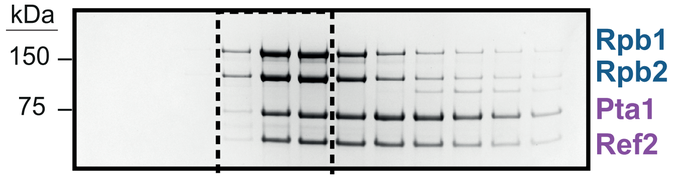
Carminati et al, bioRxiv

Pol II CTD is not required



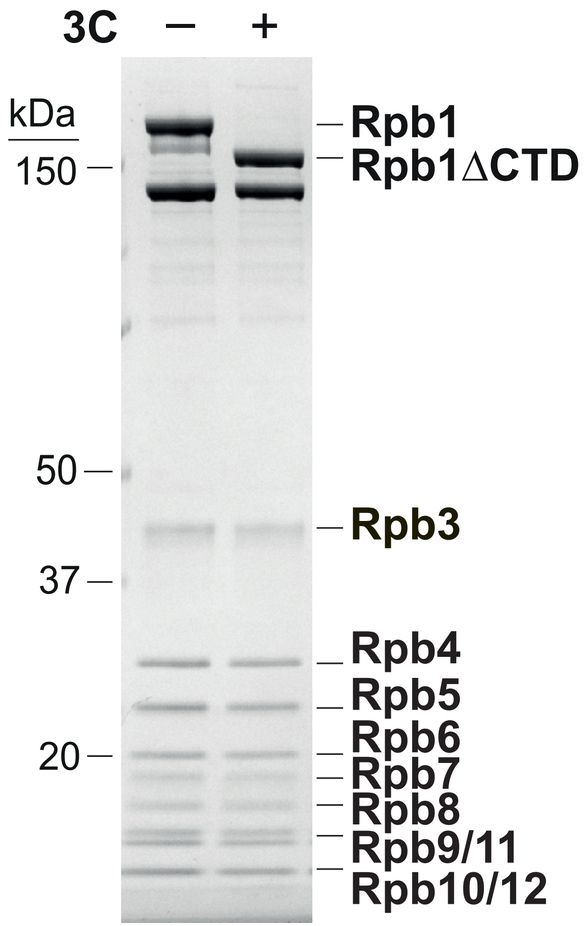


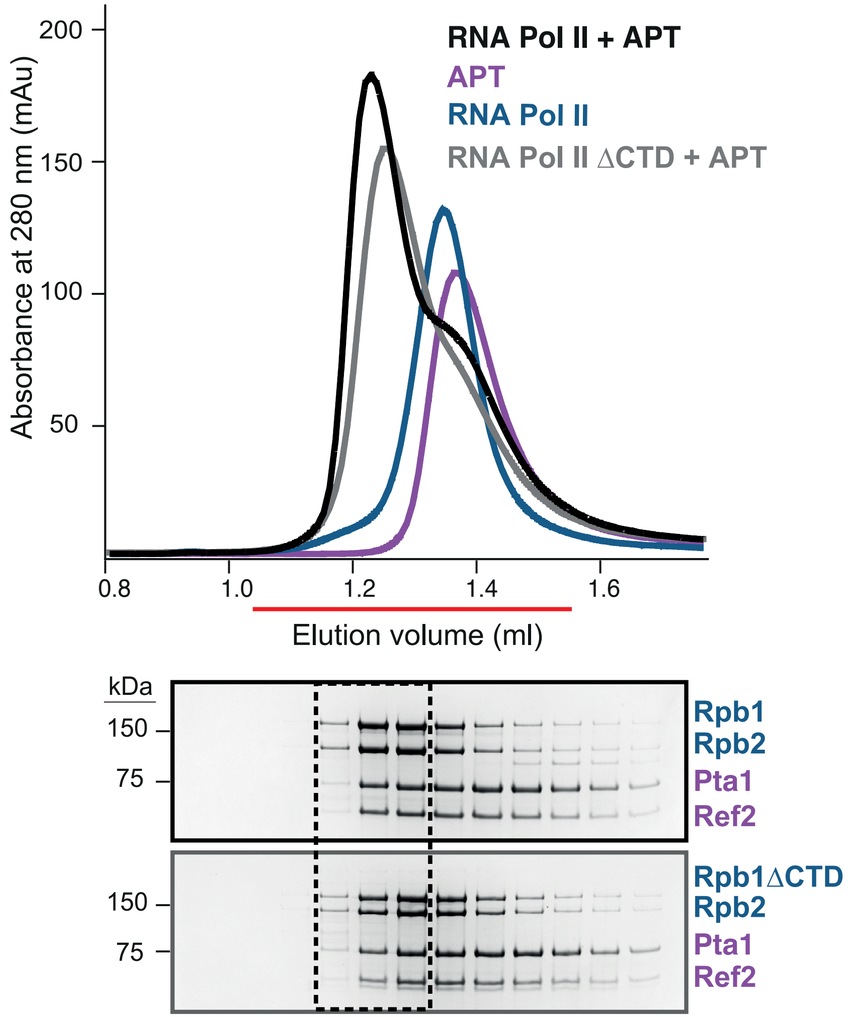




Carminati et al, bioRxiv

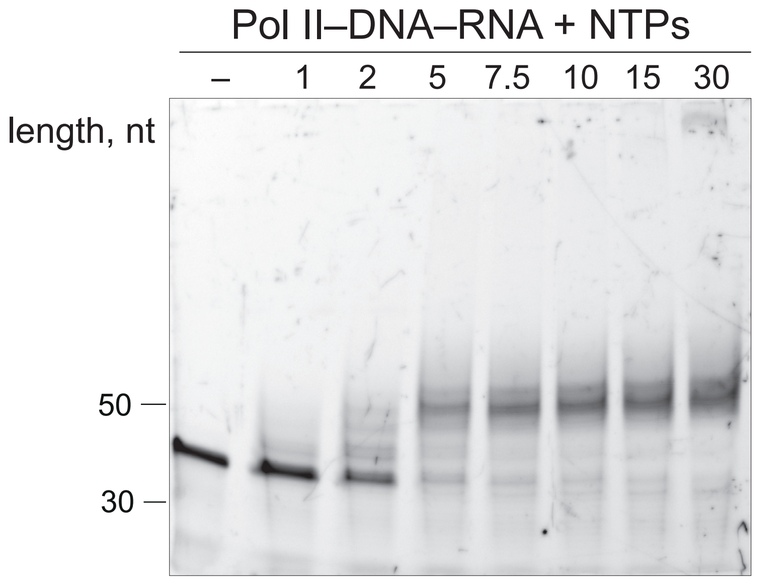
Pol II CTD is not required





Carminati et al, bioRxiv

In vitro transcription is compatible with APT/CPF



Carminati et al, bioRxiv

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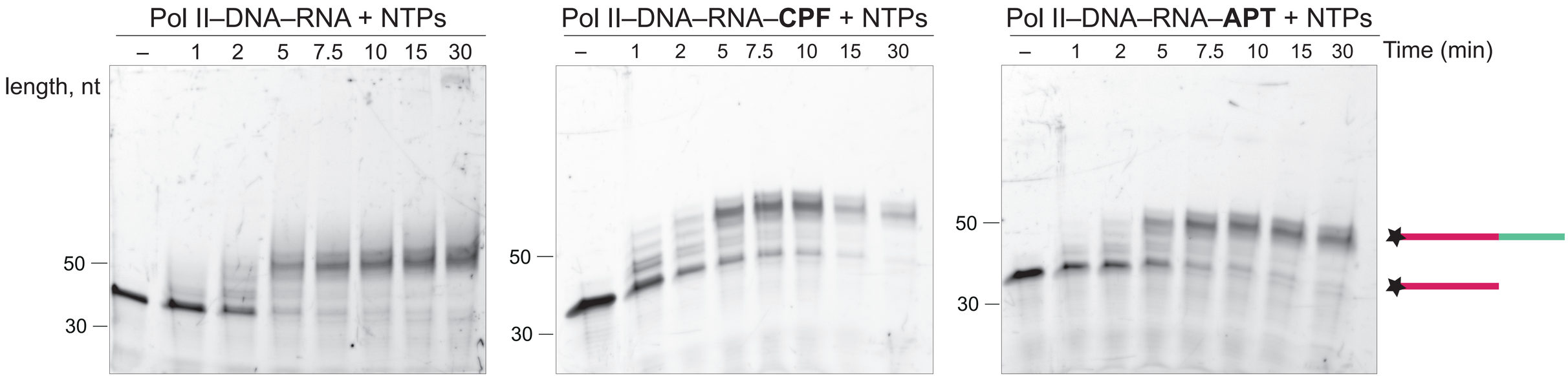
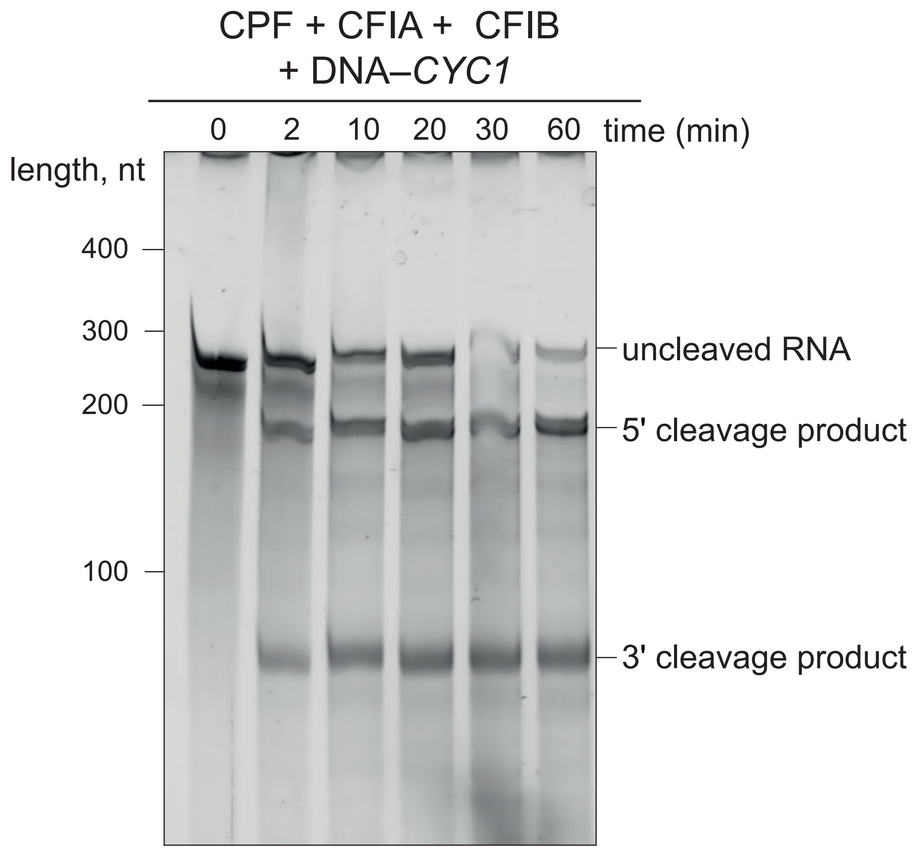


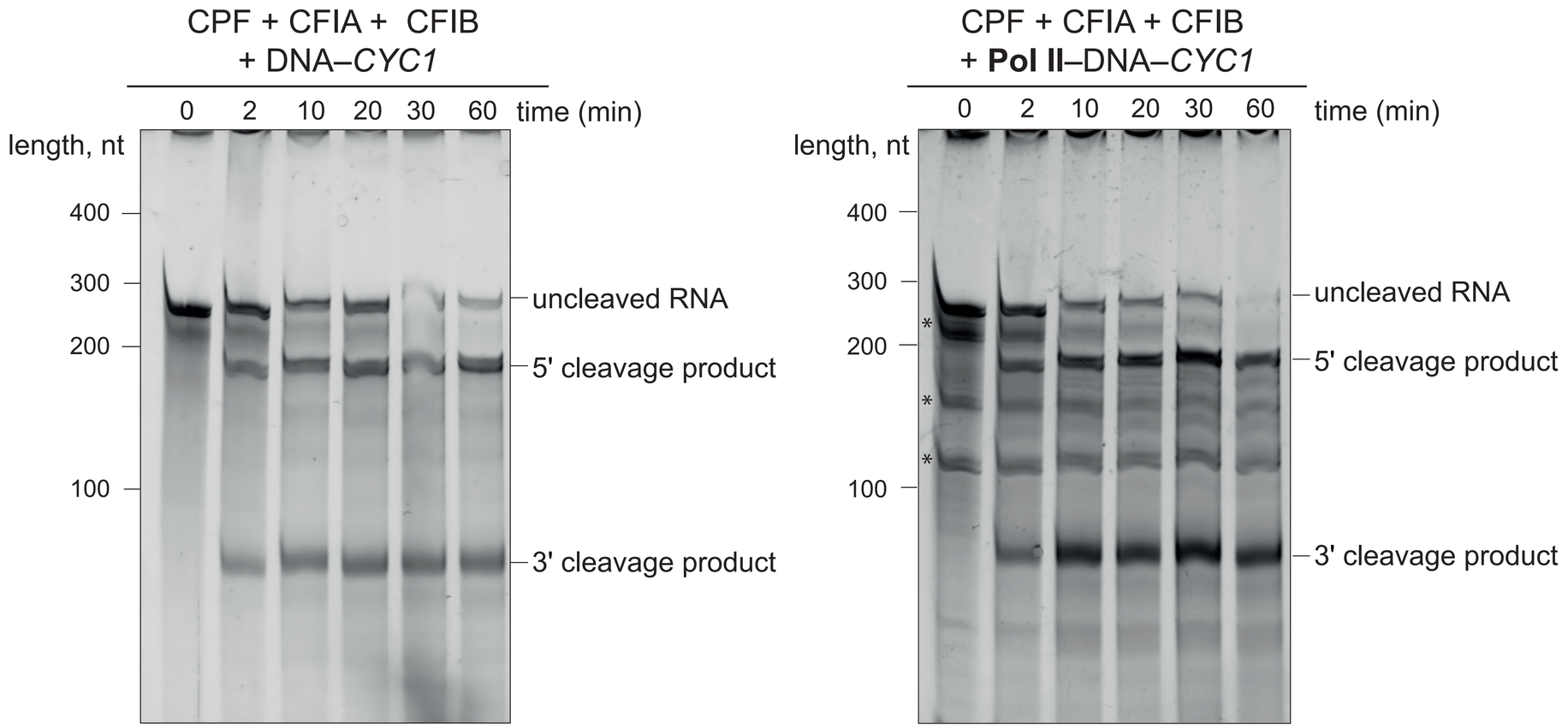
Carminati et al, bioRxiv

CPF is not stimulated by Pol II



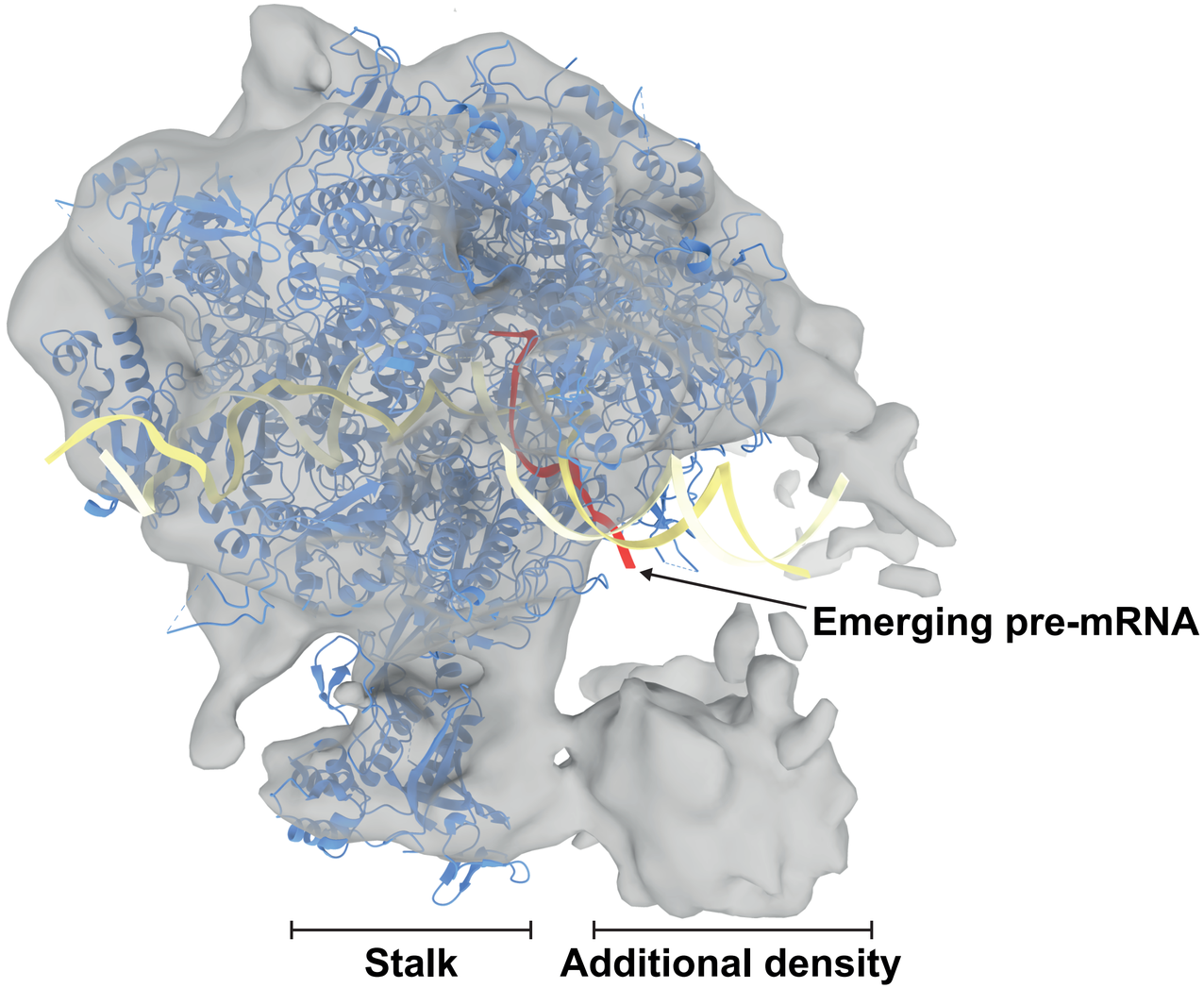
Carminati et al, bioRxiv

CPF is not stimulated by Pol II

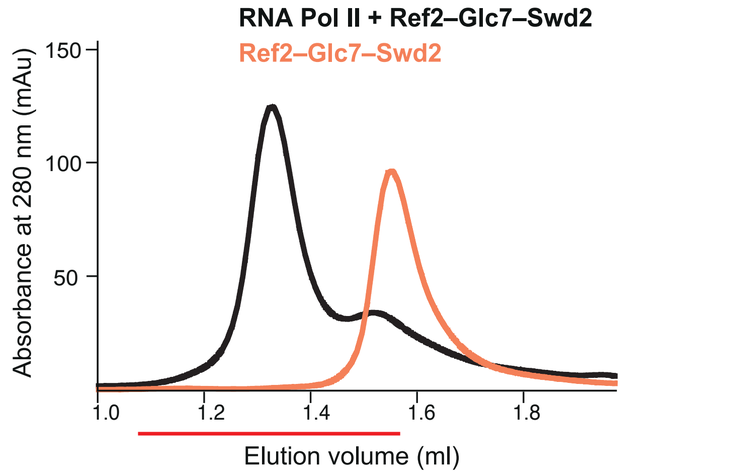


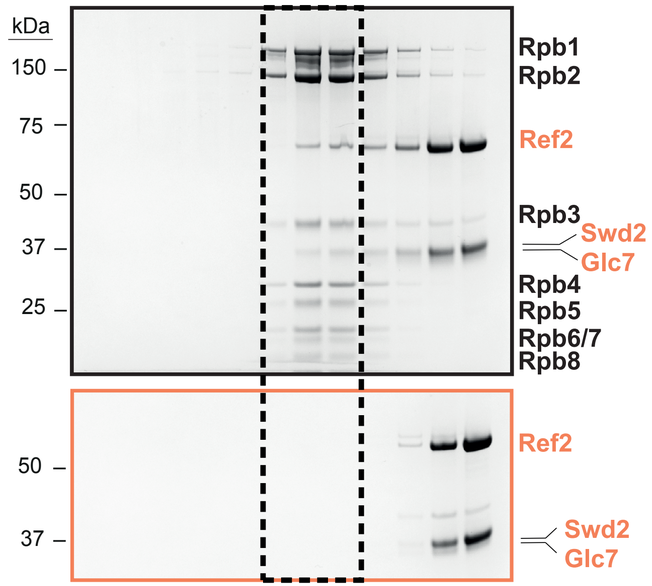
Carminati et al, bioRxiv

APT interacts directly with Pol II



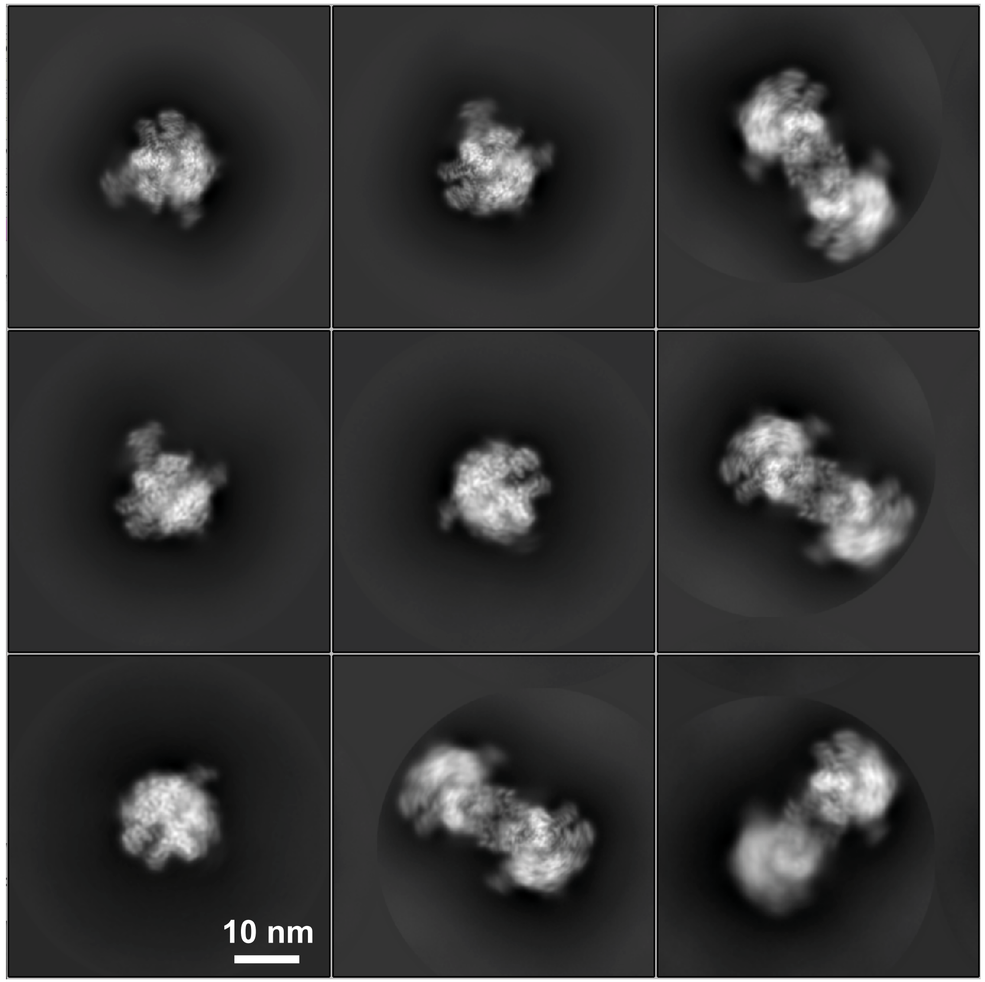
Ref2-Glc7-Swd2 sub complex also interacts with Pol II





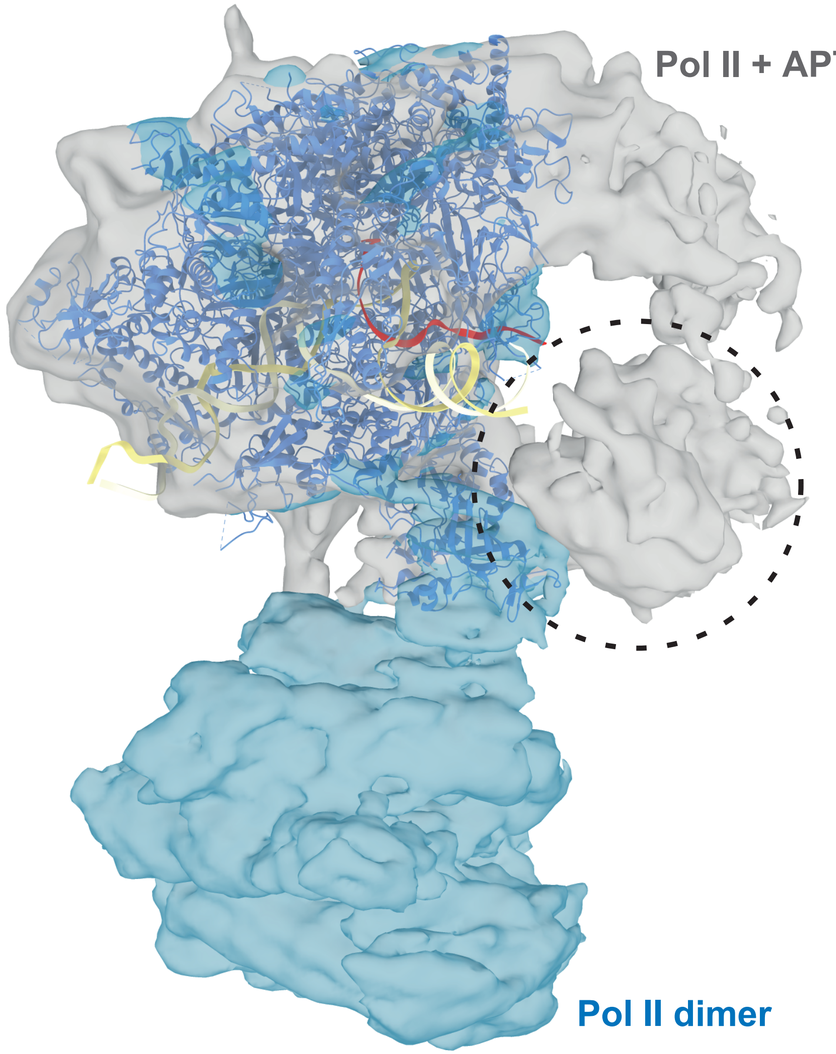
Carminati et al, bioRxiv

CPF promotes dimerisation of Pol II



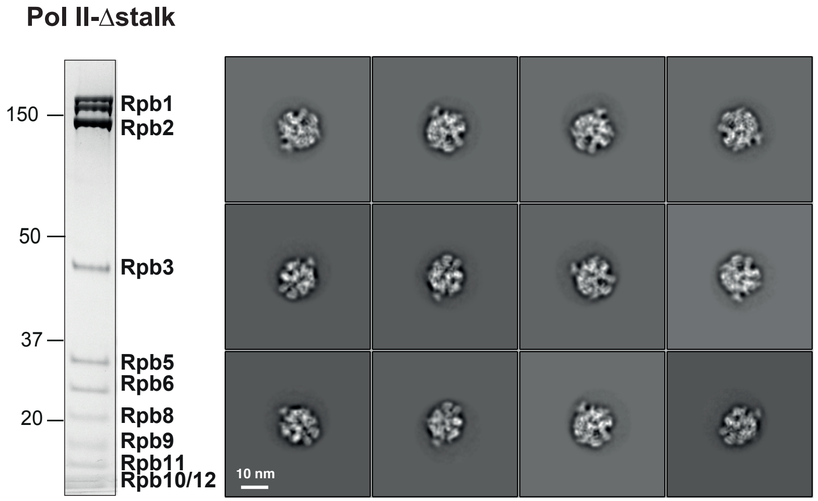
Carminati et al, 2022 bioRxiv

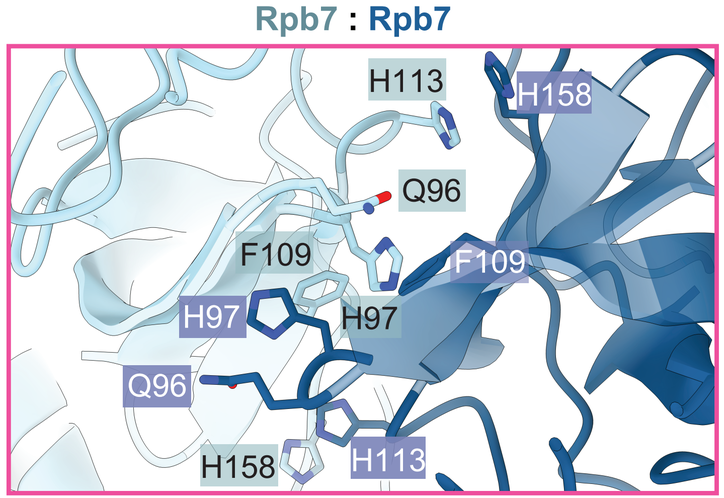
The Pol II dimer is compatible with APT binding



Carminati et al, bioRxiv

The Pol II stalk is required for dimer formation





Carminati et al, bioRxiv

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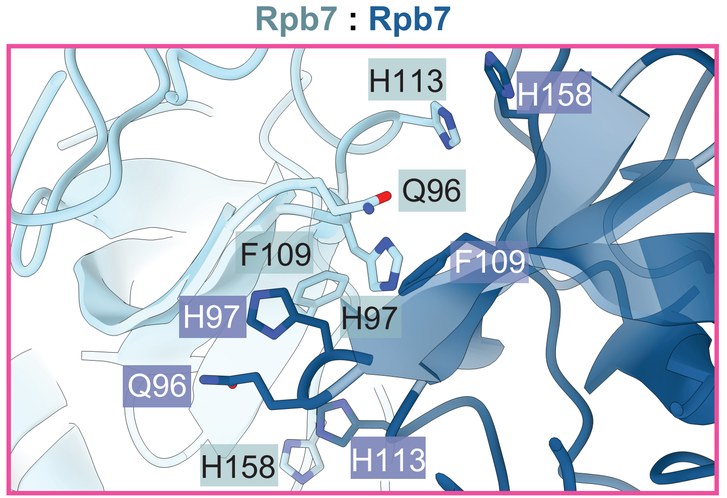
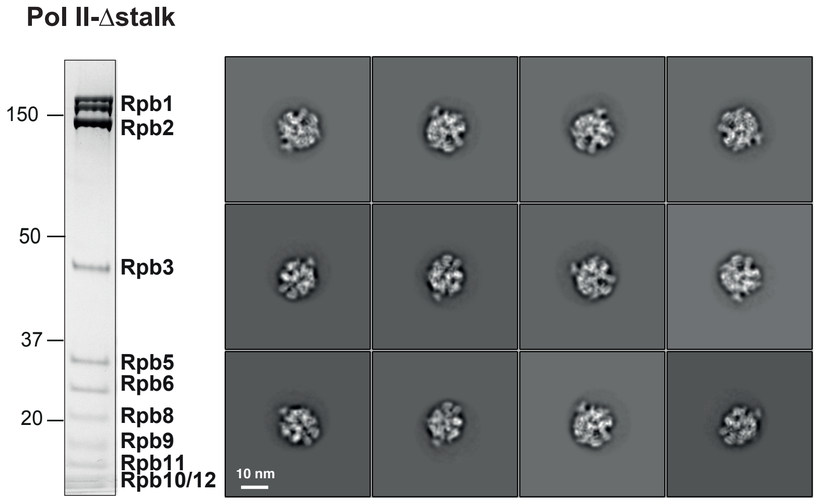
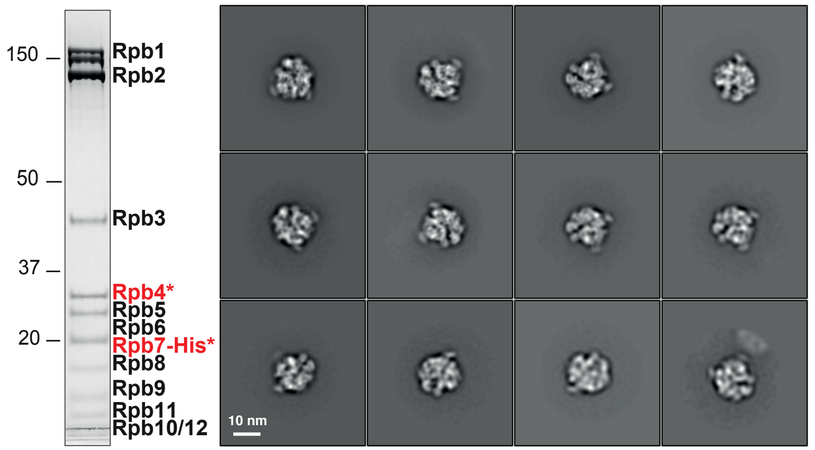
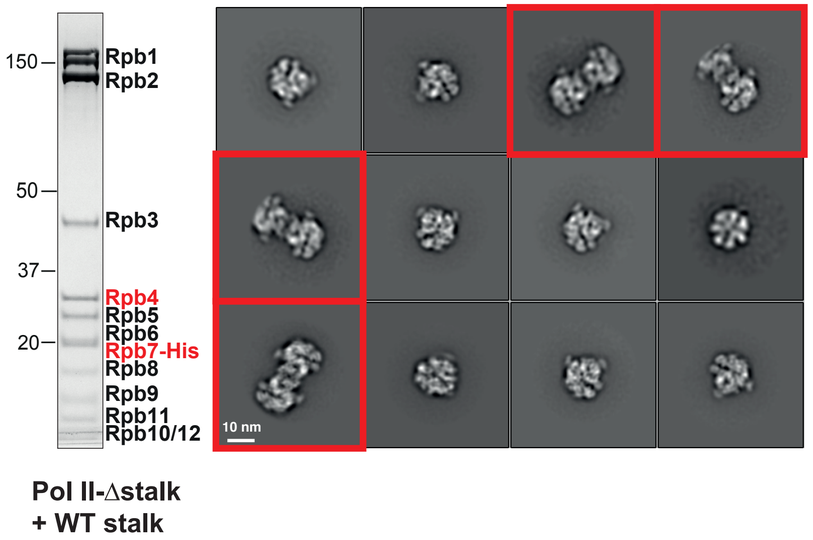


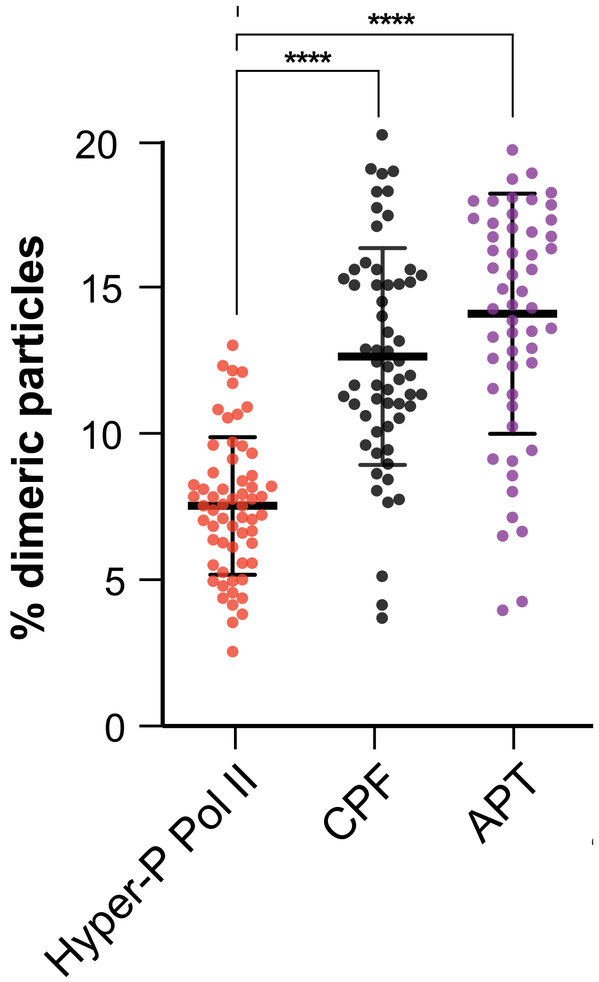






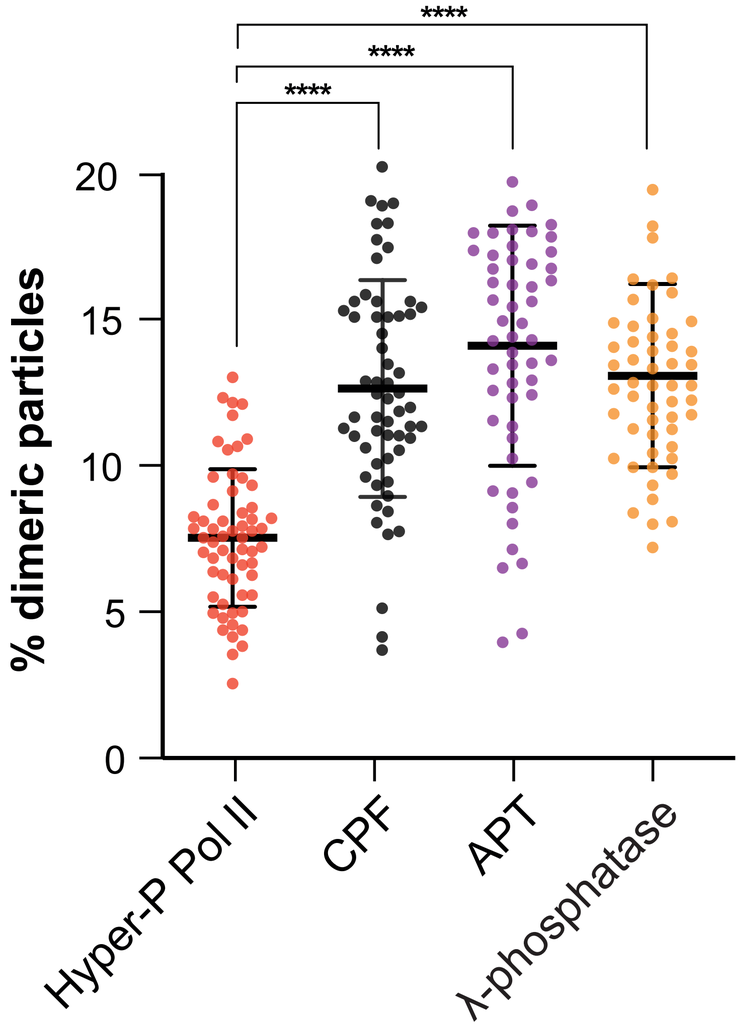
Carminati et al, bioRxiv

CTD dephosphorylation promotes Pol II dimerization



Carminati et al, bioRxiv

CTD dephosphorylation promotes Pol II dimerization



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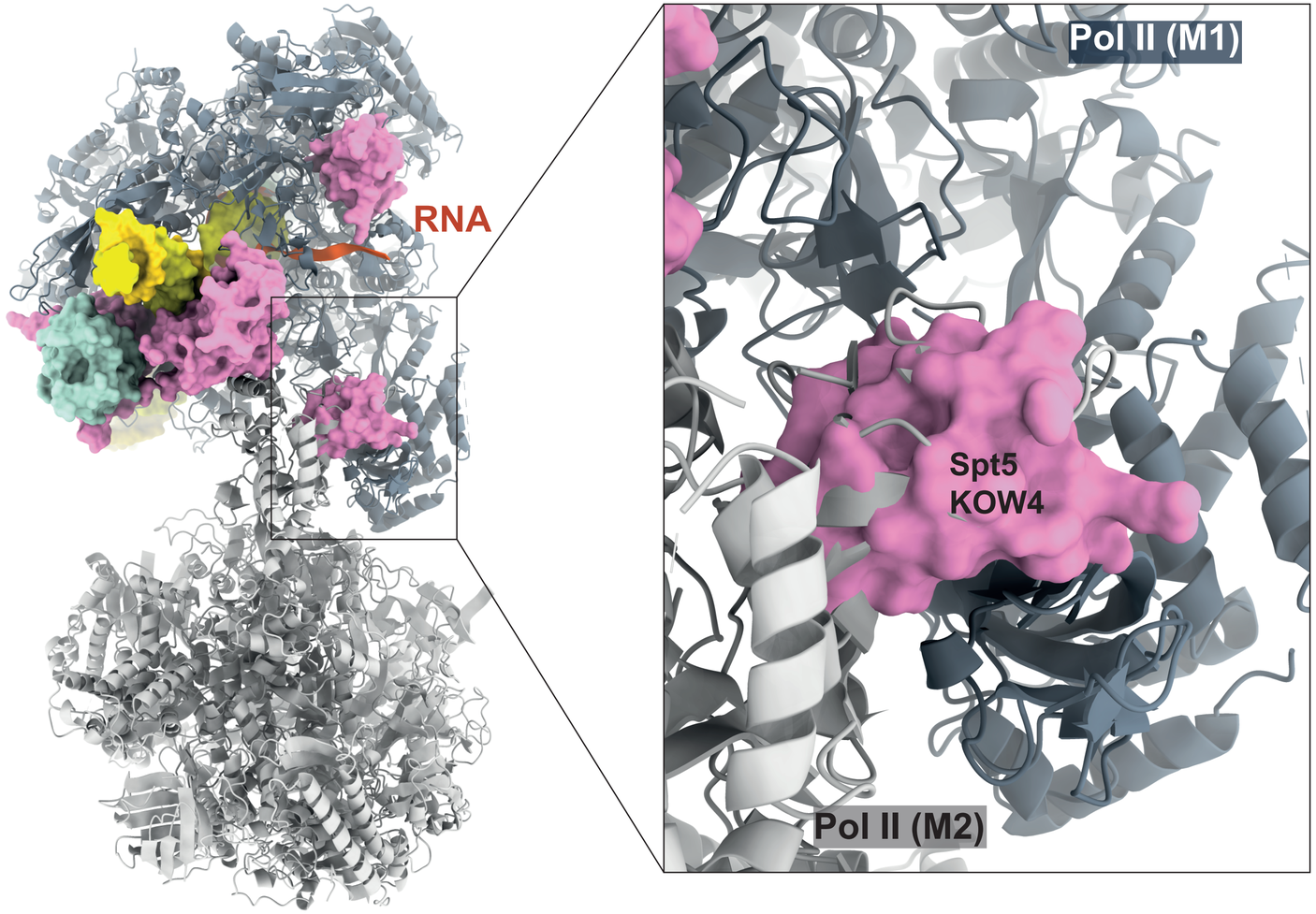
The Pol II dimer clashes with transcription factors



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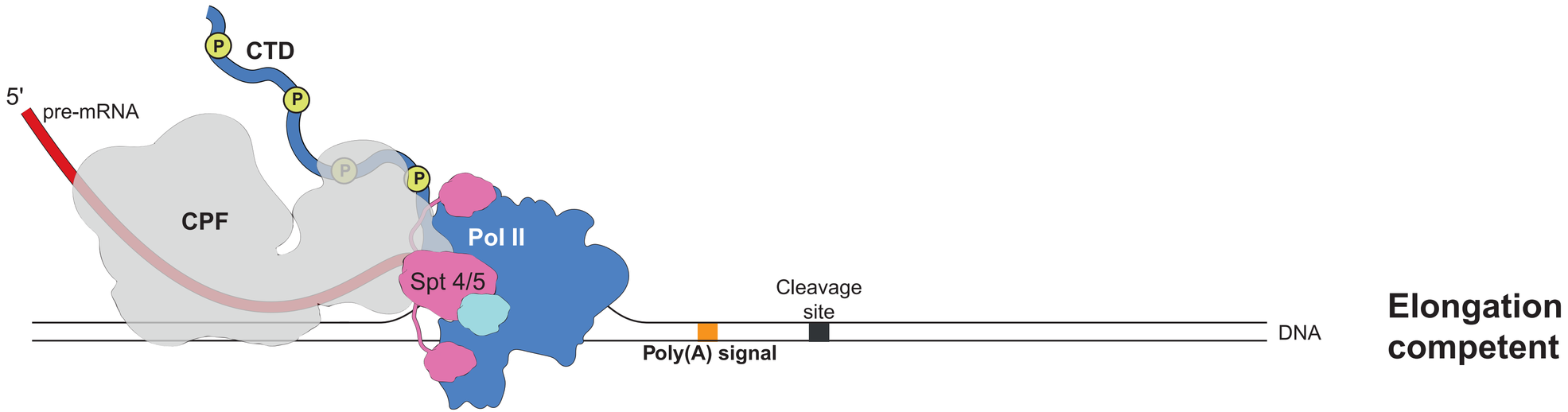
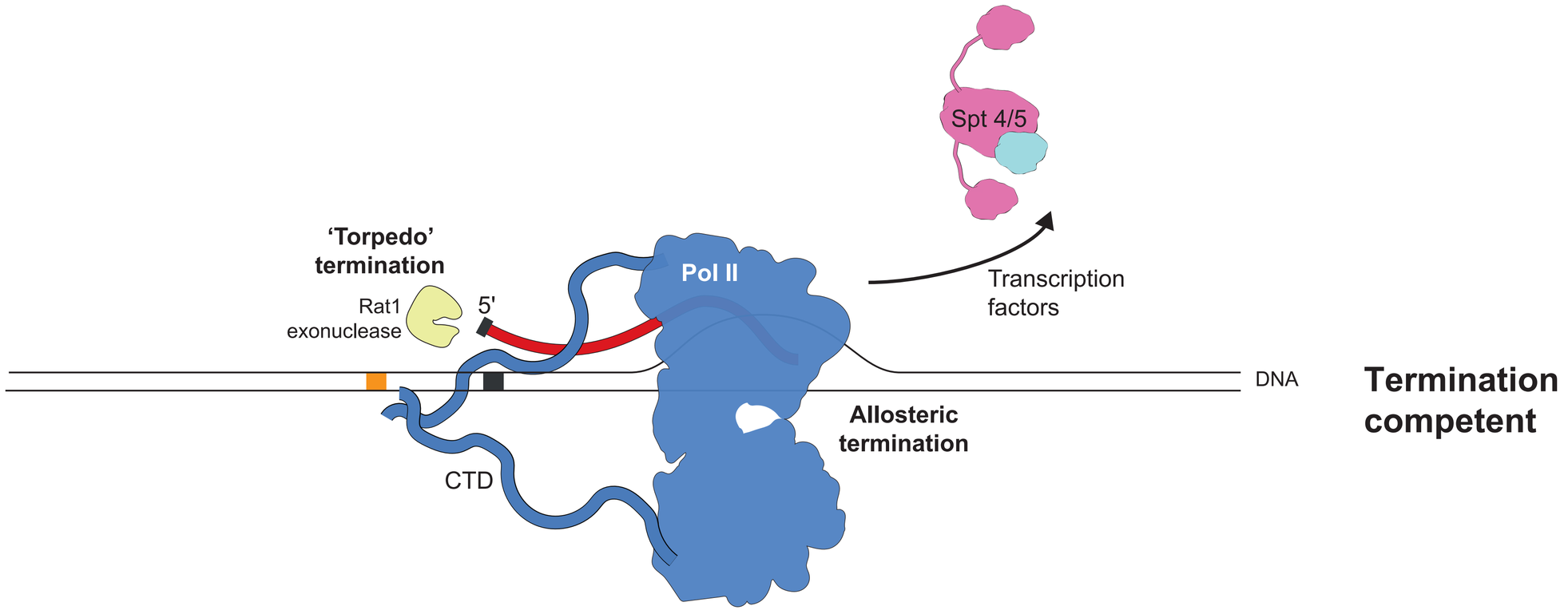
Model for transcription termination



Carminati et al, bioRxiv

Model for transcription termination



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